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# Canadian Journal of Research

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VOL. 14, SEC. C. AUGUST, 1936 NUMBER 8


## FROST-HARDENING STUDIES WITH LIVING CELLS

### II. PERMEABILITY IN RELATION TO FROST RESISTANCE AND THE SEASONAL CYCLE[1]


BY J. LEVITT[2] AND G. W. SCARTH[3]



### Abstract

Cell permeability as estimated by the plasmolytic method in various types of plants and for different classes of solute has been studied in relation to frost resistance and the seasonal cycle. Permeability is found to increase greatly with hardening, whether induced by low temperature or other conditions, and it seems to parallel closely the seasonal changes in frost resistance. As regards different species and varieties, cell permeability in the hardened state shows better correlation than any other character with ability to resist frost. The permeability change is greatest towards potassium nitrate—at least in cells (*viz.*, those of hardy woody plants) that are definitely permeable to the salt; the change is more moderate towards polar non-electrolytes with small molecules, such as urea, but with these it occurs in all plants capable of hardening; towards apolar substances, such as urethane, there is no change. These relations point to a widening of the aqueous pores or increased hydration of the plasma membrane as the mechanism of the permeability increase. Hypotheses are put forward as to the means by which freer permeability to water may increase resistance to certain types of mechanical injury by frost.


Many investigators who have attacked the problem of hardiness by the usual physico-chemical methods have been forced eventually to conclude that the seat of the major resistance is the living protoplasm. Indeed, some have regarded their results as proving that hardiness is attended by a protoplasmic change; but the validity of such an inference is doubtful because an alteration in protoplasm can be determined with certainty only from investigation of the living cell itself and not of the products of its disorganization.

On account of the complexity and lability of protoplasm and our lack of knowledge of its true nature, few methods are available for studying its change during life. One of the measurable properties which has received a great deal of attention is permeability. It is moreover a vital property of which post-mortem study tells us nothing. Without any preconceived ideas as to whether or how this might be affected by the hardening process, the following research was undertaken—with surprising results.


[1] *Manuscript received July 8, 1936.*
*Contribution from the Department of Botany, McGill University, Montreal; with financial assistance from the National Research Council of Canada.*



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## Review of Literature

The possibility that cold injury and resistance to it may in some way be connected with permeability changes has from time to time been suggested. Yet practically no experimental work on the subject has ever been attempted. Lidforss (11) suggested that bacteria and mosses owe their high degree of resistance (in the absence of quantities of sugars) to a ready permeability for electrolytes. In this way, the high concentration of salts which, as Gorke (7) emphasized, must occur in the cell on ice formation, would be prevented by diffusion into the intercellular spaces. The experimental evidence which Lidforss offers is the fact that *Mnium cuspidatum* deplasmolyzes within two hours in a 5.5% potassium nitrate solution, and *Neckera* during 48 hours in 7% potassium nitrate, both of which he considers unusually rapid.

Pantanelli (13) found that cells of the mandarin orange *(Citrus nobilis)*, when subjected to temperatures near freezing, suffer a progressive increase in permeability. Thus, there is a rapid emission of water from the tissue if it is kept dry, or an exosmosis of substances when immersed in water. Bennett (1) recently showed that potato tissue, after being stored at low temperatures, was unable to retain its cellular contents when placed in tap water. The lower the temperature the more rapid was the loss both of electrolytes and non-electrolytes. Golus (6) has recently asserted that hardiness is accompanied by lowered permeability, basing this on the fact that exosmosis from frozen, hardened wheat seedlings was less than that from frozen unhardened ones. All these results have merely to do with changes caused by injury. Death by any method will cause cells to lose their property of semi-permeability, and the larger the number of cells killed the greater will be the increased exosmosis of the affected tissue. Dexter *et al.* (4) have, in fact, made use of this principle in determining the degree of hardiness of plant material. They first subject the plant to freezing temperatures and then determine the quantity of solutes that diffuse into distilled water by measuring its electrical conductivity.

Other suggestions with regard to permeability changes have been made, based mainly on theoretical considerations. Coville (3) believes that the effect of low temperatures in breaking dormancy may be due to an increased permeability of the membrane surrounding the starch grains, thus enabling enzymes to come into contact with them and to convert them into sugars. It has been pointed out that this theory cannot explain the reverse process.

The problem undertaken in the following investigation is to determine just what changes in permeability, if any, occur on exposure to low temperature, and whether hardy and non-hardy plants show any differences. Only when a change has once been definitely established is it in order to consider the theories suggested.

## Method and Procedure

For purposes of measurement, cellular permeability may be divided into three types according to the nature of the substances passing in or out:—permeability to *(a)* water, *(b)* non-electrolytes, *(c)* electrolytes. Of the various methods of measuring permeability, the plasmolytic alone is suitable for all three categories of substance and alone is applicable to almost any kind of plant cell. Being also reasonably rapid and capable of a sufficient degree of quantitative estimation, it has proved the most successful in the present study and is the means by which all the positive results were obtained.

In general, our procedure was to compare by means of this method the permeability of the cells of corresponding tissues in the unhardened and hardened state, respectively, and to follow changes as the plant passed from the one phase to the other. The material studied represented various degrees of cold resistance, from tender annuals to hardy trees. The complete list is as follows:

*(a)* Herbaceous plants:—*Brassica oleracea capitata, Trifolium pratense, Helianthus annuus, Ricinus communis.*

*(b)* Woody plants:—*Pyrus malus, Hydrangea paniculata, Catalpa hybrida, Picea pungens, Liriodendron tulipifera, Caragana arborescens, Aesculus hippocastanum, Pterocarya rhoifolia.*

The moderately hardy herbaceous species were hardened and tested in a cold chamber. To attain the cold-resistant state the seedlings were exposed to a temperature of 5° C. for five or more days in the presence of artificial lighting. A 12-hour period at about −5° C. was employed to test their hardiness.

The highly resistant trees and shrubs, besides being subjected to artificial conditions, were also examined as they passed through their seasonal changes in a state of nature. Measurements were made principally on the pith and chlorenchyma cells from the petioles of herbaceous plants, and on the cortical cells of woody plants. For purpose of comparison, cells of the same type and position were employed.

The *osmotic value* of the cells was first determined (9). *Permeability* was then studied on the same preparations. Penetration of both water and solutes was measured; and among the latter were examples of various degrees of polarity, from inorganic salts to weakly polar organic compounds. The results are classified under permeability to (1) water, (2) salts (potassium nitrate), (3) polar non-electrolytes (urea, glycerol, etc.), and (4) non-polar substances.

The nature of our problem involved certain complications which in most comparisons of permeability are negligible. Particularly the wide differences between the osmotic values of the hardened and unhardened cells made it impossible to use the same plasmolyzing solutions and also have the cells pass through the same changes in volume and surface in both cases. We have found, however, that the concentration of the plasmolytic has little effect on permeability (see (10) and later in this paper). Hence, without vitiating the experiment, the solution could be varied so as always to give equal degrees of plasmolysis. In the case of water permeability this was the standard procedure and, since cells of about equal size are compared, the absorbing surface should pass through the same area change in both. However, it must be admitted that differences in the amount of non-solvent space will complicate matters still further (9).

In the case of those solutes which penetrate very slowly relative to water, the penetration expressed in *mols* per hour was calculated from the deplasmolysis time and the average difference between internal and external concentration of penetrant. This, of course, is not an exact mode of calculation, but owing to the number of complications and sources of error it seemed difficult and of doubtful value to employ an integration formula on our data. The simple formula used was derived as follows:

Let $P$ = external concentration in mols of penetrant—which remains virtually constant

$p$ = initial osmotic value of the cell, expressed as mols of penetrant

$t$ = time in hours for completion of deplasmolysis

$P.D.$ = average partial pressure (conc.) difference of penetrant (mols)

$$= P - \frac{P-p}{2} = \frac{P+p}{2} \text{ mols} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(I)}$$

Assuming that the cell is practically isosmotic with the solution at deplasmolysis, the concentration of penetrant in the cell is then $P-p$. Therefore, as a standard of comparison, the amount of substance which would enter a cell in one hour under a $P.D.$ of 1 mol

$$= \frac{P-p}{t \times P.D.} \text{mols} = \text{(from (1))} \frac{2(P-p)}{t(P+p)} \text{ mols.}$$

This is the value used as an expression of permeability.

In most of the determinations of permeability to *solutes*, differences in degree of plasmolysis were not eliminated, but a preliminary test as well as theoretical considerations seem to indicate that in the type of material used this disturbing factor tends to be inoperative.

In the test, the rate of deplasmolysis of similar cells in solutions of *different concentrations* was measured and permeability calculated according to the above formula, that is without reference to surface area. The results are shown in Table I. The values for each species are nearly constant in spite of great differences in degree of plasmolysis.

In explanation of this result, it is to be noted that in sections such as we used, consisting of a bundle of elongated cells, the effective surface of each and all of the cells in the interior of a section tends on an average to remain the same as deplasmolysis proceeds, for the reason that all the protoplasts expand at the same rate and blanket one another. Thus, whatever concentration of plasmolytic is used, the area of absorbing surface is the same in each case and is virtually constant throughout the test. Accordingly, the results are taken as justifying the application of the formula used. Along with those in Table II they also indicate that varying the concentration of urea does not greatly modify permeability.

TABLE I

COMPARISON OF THE PERMEABILITY RATE IN SOLUTIONS OF DIFFERENT CONCENTRATIONS

| Species | Conc. urea, mols | Conc. thiourea, mols | O.P., atm. | Deplas., min. | Perm.,* mols per hr. |
|---|---|---|---|---|---|
| Cabbage | | 0.6 | 12.4 | 22 | 0.44 |
| | | 0.7 | | 36 | 0.52 |
| | | 0.8 | | 60 | 0.44 |
| *Aesculus* | 0.93 | | 12.4 | 86 | 0.41 |
| | 1.86 | | | 142 | 0.48 |
| | 2.79 | | | 165 | 0.50 |
| *Catalpa* | 0.93 | | 14.9 | 64 | 0.38 |
| | 1.86 | | | 107 | 0.56 |
| | 2.79 | | | 151 | 0.51 |
| *Hydrangea* | 1.86 | | 19.3 | 27 | 1.77 |
| | 2.79 | | | 35 | 1.90 |
| *Picea* | 1.86 | | 22.5 | 110 | 0.36 |
| | 2.79 | | | 127 | 0.47 |

**Calculated per mol difference in concentration.*

An important point to discover is whether it is normal permeability which is measured or an abnormal permeability affected by the mechanical action of plasmolysis or deplasmolysis on the protoplasm. Conceivably it might be resistance to plasmolysis that changes with hardening, rather than permeability *per se*.

The mechanical effect of plasmolysis and deplasmolysis on permeability has been found to vary with the type of cell and its condition at the moment

(5, 8, 14). Accordingly, we tested some of our material that showed the greatest change in permeability. Comparison of penetration of urea in hyper- and hypotonic solutions was made by a modification of the method of Huber and Schmidt (8) on three species in their hardened state.

TABLE II

EFFECT OF PLASMOLYSIS ON PERMEABILITY

| Species | O.P., atm. | Time in minutes | | Penetration rate, mols per hr. | |
|---|---|---|---|---|---|
| | | Plasmolyzed | Unplasmolyzed | Plasmolyzed | Unplasmolyzed |
| | | 0.8 *M* thiourea | 0.4 *M* thiourea | | |
| Cabbage | 12.4 | 60 | 100 | 0.44 | 0.40 |
| | | 1.5 *M* urea | 0.5 *M* urea | | |
| *Hydrangea* | 22.5 | 52 | 56 | 0.54 | 0.71 |
| *Catalpa* | 22.5 | 75 | 90 | 0.38 | 0.44 |

Two parallel determinations were made; one in the ordinary way, the other by use of a hypotonic solution. In the latter case, since no plasmolysis occurred, a special procedure was necessary to find out how much solute penetrated per unit time. Huber and Schmidt (8) determine the increase in osmotic pressure by plasmolysis in dextrose after a definite time in the penetrating solution. In our tests, instead of using dextrose we employed a mixture composed of a partial pressure of calcium chloride equal to the original concentration of the cell sap plus a partial pressure of urea equal to half the concentration of the urea in the hypotonic penetrating solution. A section was from time to time removed from the latter and placed in this composite solution. If the cells plasmolyzed, the section was discarded and a few minutes later another was removed from the hypotonic penetrating solution and similarly tested. This procedure was repeated until the time when no plasmolysis occurred. At this point, the concentration of urea in the cells (which had never been plasmolyzed) was equal to half that in the penetrating solution and exactly equal to the amount in the composite solution. The purpose of this modification of the method used by Huber and Schmidt was to prevent exosmosis of urea during the determination of osmotic pressure. In the case of cabbage, thiourea was employed.

The results are given in Table II. Permeability was found to be practically unaffected by plasmolysis in all three species. Apparently, then, our deplasmolysis tests, in the case of solutes like urea at least, are a measure of normal permeability. On the other hand, the more rapid deplasmolysis involved in the usual test of *water penetration* does produce a temporary increase of permeability (10). Differences in the operation of this effect have to be taken into account in making comparisons of water permeability (see later).

## Results

1. *Permeability to Water*

Cabbage seedlings were hardened in the cold chamber in the usual way and determinations of water permeability made from time to time. For reasons explained above, the standard procedure was to plasmolyze for 15 to 20 minutes in twice isotonic dextrose and then to deplasmolyze in an isotonic solution of the same substance. The values given for relative permeability take account of the pressure difference as well as deplasmolysis time.

TABLE III

WATER PERMEABILITY OF CABBAGE SEEDLINGS AT DIFFERENT STAGES OF HARDENING. EACH VALUE AN AVERAGE OF 3 PLANTS

| Hardening, days | O.P., atm. | Deplasmolysis time, min. | Relative permeability* |
|---|---|---|---|
| 0 | 10.6 | 15 | 2.1 |
| 3 | 12.4 | 8 | 3.4 |
| 7 | 16.1 | 5 | 4.1 |
| 10 | 16.1 | 5 | 4.1 |

*$Relative\ permeability = \frac{10{,}000}{t\ secs. \times av.\ P.D.}$

Table III shows that the water permeability increases on hardening until the end of a week, and then remains constant. It is then double that of the unhardened plants.

*Woody plants.* Fully hardened twigs were taken indoors during midwinter and then left standing in water at room temperature for a few weeks. Table IV shows that during this time—that is, in the period when hardiness was lost—a marked decrease in permeability to water occurred.

TABLE IV

WATER PERMEABILITY BEFORE AND AFTER LOSS OF HARDINESS. PLASMOLYZED 15 MINUTES IN 2*i* $CaCl_2$; DEPLASMOLYZED IN $\frac{1}{4}$ $CaCl_2$. JANUARY 16, 1935

| Days at room temp. | *Hydrangea* | | | *Catalpa* | | |
|---|---|---|---|---|---|---|
| | O.P., atm. | Deplas., sec. | Rel. perm. | O.P., atm. | Deplas., sec. | Rel. perm. |
| 0 | 23.9 | 20 | 21 | 22.5 | 35 | 12.7 |
| 37 | 11.2 | 120 | 7.5 | 16.1 | 85 | 7.3 |

Experiments with woody plants under natural hardening conditions were begun before standardization of the procedure. In this case the cells were plasmolyzed for 15 minutes in a 2*M* dextrose solution and deplasmolyzed in a *M*/2 dextrose solution (except in *Caragana*, for which, on account of its high cell-sap concentration, double strength was used, *i.e.*, *M* dextrose). But since the change in osmotic pressure was small during the period when the determinations were made, the differences in degree of plasmolysis were relatively slight. Relative permeability was calculated as before. Table V indicates a definite increase in permeability to water during the late fall. From then on, the rate remained constantly high—that is, deplasmolysis was so rapid as to be immeasurable by the technique used.

TABLE V

WATER PERMEABILITY OF WOODY PLANTS DURING DIFFERENT STAGES OF NATURAL HARDENING. PLASMOLYZED 15 MIN. IN 2*M* DEXTROSE; DEPLASMOLYZED IN $\frac{M}{2}$ DEXTROSE, EXCEPT *Caragana* WHICH WAS DEPLASMOLYZED IN *M* DEXTROSE

| Date | *Picea* | | | *Liriodendron* | | | *Caragana* | | | *Catalpa* | | | *Hydrangea* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | O.P., atm. | Depl., sec. | Rel. perm. | O.P., atm. | Depl., sec. | Rel. perm. | O.P., atm. | Depl., sec. | Rel. perm. | O.P., atm. | Depl., sec. | Rel. perm. | O.P., atm. | Depl., sec. | Rel. perm. |
| Oct. 10 | 21.2 | 210 | 2.0 | 18.6 | 80 | 5.6 | 32.3 | 60 | 10.3 | 31.6 | 300 | 1.2 | 16.8 | 360 | 1.3 |
| Oct. 31 | 28.7 | 30 | 12.1 | 21.9 | 60 | 6.9 | 29.5 | 40 | 16.9 | 26.6 | 45 | 8.4 | 20.6 | 180 | 2.4 |
| Nov. 21 | 25.2 | 60 | 6.5 | 21.2 | 30 | 14.0 | 31.6 | 30 | 21.1 | 22.5 | 180 | 2.3 | 22.5 | 270 | 1.5 |
| Dec. 12 | 24.6 | 30 | 13.1 | 23.9 | 30 | 13.3 | 29.5 | * | * | 22.5 | 60 | 6.9 | 20.6 | 150 | 2.9 |

**Too rapid for measurement.*

These preliminary tests indicate that in order to measure the water permeability of fully hardened cells, some method of slowing up the rate of deplasmolysis is essential. Since permeability of *Catalpa* cells was found to have a temperature coefficient of about 2.1 (Table VI), an obvious way of reducing the rate was to lower the temperature at which the test was conducted.

TABLE VI

TEMPERATURE COEFFICIENT OF WATER PERMEABILITY OF *Catalpa* CELLS

| | O.P. | Deplasmolysis time (secs. from 2*i* to $\frac{1}{2}$ $CaCl_2$) | | | Temperature coefficient | |
|---|---|---|---|---|---|---|
| | | 22° C. | 10° C. | 0° C. | 0–10° C. | 10–22° C. |
| Hardened | 18.6 | 40 | 105 | 225 | 2.14 | 2.20 |
| Unhardened | 11.2 | 100 | 255 | 525 | 2.06 | 2.13 |

For this purpose, a special low-temperature stage was made, by means of which the permeability could be measured at 0° C. Fig. 1 shows its construction.

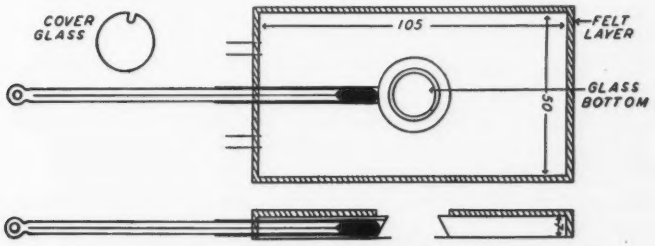


FIG. 1. *Low temperature stage used for measuring water permeability at 0° C. Dimensions in mm.*

The permeability was then measured at intervals from March to June. The standard procedure of plasmolyzing the cells in 2*i* dextrose and deplasmolyzing in $\frac{1}{2}$ dextrose was followed. There was no pretreatment with neutral red and calcium chloride, for preliminary tests showed that this causes a marked decrease in permeability. In other words, the true rate was found to be actually greater than the high values already recorded! Furthermore, the difference between hardy and non-hardy cells is undoubtedly greater than that already found. Thus, Lucké and McCutcheon (12) demonstrated the same retarding effect of calcium, and they further found that this effect only occurred in the case of cells with high permeability and not with those already having a naturally low rate.

Table VII, recording seasonal change, shows a decided drop in permeability during spring. In March and April the rate of deplasmolysis is so rapid as to be practically instantaneous, even at 0° C. In May, however, the time required is a minute or more.

TABLE VII

SEASONAL DETERMINATIONS OF PERMEABILITY TO WATER AT 0° C. DEPLASMOLYSIS TIME (SEC.) FROM 2*i* DEXTROSE TO $\frac{1}{2}$ DEXTROSE

| Date | *Hydrangea* | *Catalpa* | *Caragana* | *Liriodendron* |
|---|---|---|---|---|
| Mar. 12 | 30 | 60 | 30 | 30 |
| Mar. 31 | 15 | 75 | 20 | 20 |
| April 20 | 15 | 60 | 15 | 30 |
| May 11 | 60 | 180 | 60 | 90 |

However, a troublesome though interesting complication arose in these investigations. During April and May, as the plants lost in hardiness, it became increasingly difficult to distinguish deplasmolyzing cells, owing to the fact that more and more of the cells burst during deplasmolysis. A test conducted on June 1st was a failure, since all the cells burst before deplasmolysis was complete (except in the case of *Catalpa*). Yet the same nonhardy cells were for the most part uninjured when the deplasmolysis was carried out at room temperature, in spite of the fact that permeability and therefore deplasmolysis rate is about five times as great at the higher temperature! This indicates that the viscosity of the protoplasm is considerably increased by the decrease in temperature—presumably more so in the unhardened than in the hardened cells in view of the lack of injury to the latter during deplasmolysis. This question will receive treatment in a subsequent paper.

Since the permeability of the cell is increased during deplasmolysis, owing to stretching of the membrane (10), the result is to over-estimate the natural permeability. And since the deplasmolysis injury is far more pronounced in unhardened cells, the over-estimation will be greater in the case of these, and, consequently, the true difference in permeability between hardened and unhardened cells is probably much larger than the tests indicate.

On account of the complications involved in the methods so far used, the relation between water permeability and hardiness is not yet completely worked out. Some more satisfactory procedure will have to be evolved in order to get accurate results. In the meantime, we have followed more intensively the penetration of solutes, accurate measurement of which is more easily made.

2. *Permeability to Salts (Potassium nitrate)*

While no evidence of penetration of calcium chloride was found under any conditions, results were obtained with other salts. Potassium nitrate was chosen for the comparative studies.

With cabbage cells the rate of entry of potassium nitrate was too slow to give reliable results. Woody plants in their winter state, however, proved remarkably permeable to the salt, and it was found that after twigs of such

had been kept seven days at room temperature, so as to become partially dehardened, the permeability fell to a small fraction of its original level (Table VIII).

TABLE VIII

PERMEABILITY TO $KNO_3$ OF TWIGS BEFORE AND AFTER LOSS OF HARDINESS

Twigs collected February 6, 1935. (*Picea* in .75*M* $KNO_3$)

| Species | Days at room temp. | O.P., atm. | Deplas., in 1.25*M* $KNO_3$, min. | Permeability, mols per hr. |
|---|---|---|---|---|
| *Hydrangea* | 0 | 22.5 | 39 | 1.03 |
| | 7 | 19.3 | 73 | .68 |
| *Catalpa* | 0 | 22.5 | 39 | 1.03 |
| | 7 | 21.9 | 215 | .20 |
| *Caragana* | 0 | 34.6 | 16 | 0.69 |
| | 7 | 21.2 > | 720 | < .06 |
| *Liriodendron* | 0 | 25.9 | 7 | 4.50 |
| | 7 | 23.9 | 106 | .36 |
| *Picea* | 0 | 21.2 | 5 | 3.27 |
| | 7 | 23.2 | 58 | .67 |

*Seasonal changes in potassium nitrate permeability.* These results were followed up by a seasonal determination of potassium nitrate permeability on five ornamental plants and four apple varieties throughout the year. Unfortunately it was not realized at this time how greatly the prestaining treatment of sections in solutions containing calcium chloride reduced permeability. Consequently, all the results here recorded

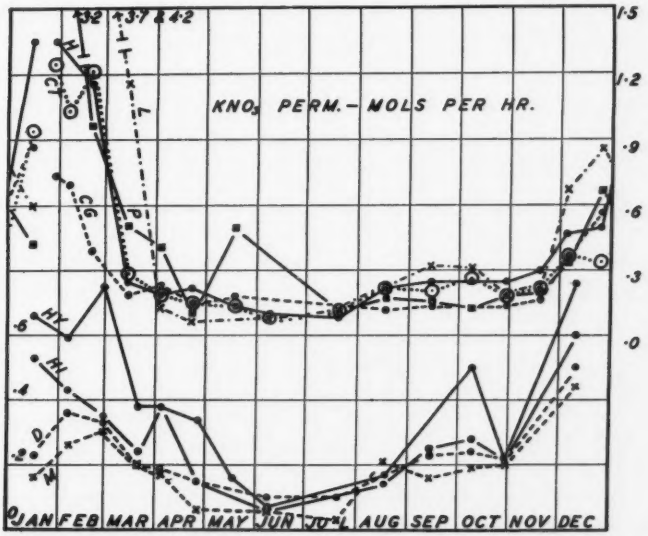


FIG. 2. *Seasonal changes in potassium nitrate permeability of hardy woody plants, determined on cortical cells of current growth. Jan. 1935–Jan. 1936.*

*Upper Curves*
*CG = Caragana arborescens*
*L = Liriodendron tulipifera*
*CA = Catalpa hybrida*
*H = Hydrangea paniculata*
*P = Picea pungens*

*Lower Curves (apple varieties)*
*HI = Hibernal*, *HY = Hyslop* } *Hardy*
*M = Milwaukee*, *D = Delicious* } *Less hardy than above*

are too low, higher rates of penetration being undoubtedly most reduced. Furthermore, fluctuations occur, since they are not all reduced to the same extent, for this will vary with the length of time in the calcium chloride solution. Nevertheless, the seasonal changes are striking (Fig. 2). The maximum occurs in all species from December to February, the minimum from May to July. Furthermore, the maximum may be many times as great as the minimum. The fact that non-solvent space $x$ (see (9) ) was not taken into account in our formula cannot greatly influence these results, for even if $x$ is assumed to be 40% in the hardy and 0% in the non-hardy cells, our maxima will only be overestimated about 67%.

As regards varietal difference, Fig. 2 shows that during winter the two hardy varieties (Hibernal and Hyslop) have much higher permeabilities than the more tender ones (Milwaukee and Delicious). It is interesting to note that of the two hardy varieties, Hyslop possesses the higher permeability, whereas Hibernal has the advantage in osmotic pressure, hardiness being about the same in both.

As a further test, ten varieties of varying hardiness were chosen, and potassium nitrate permeability determined three times between mid-January and mid-February. Table IX gives the average of these determinations. It is interesting to note that in spite of the correlation exhibited between hardiness and permeability, osmotic pressure appears to be unrelated to either. The relative hardiness of the varieties was obtained from the observations made by Dr. R. F. Suit of Macdonald College on the injury caused by the test winter of 1933–34.

TABLE IX

VARIETAL FROST RESISTANCE OF APPLES AS RELATED TO $KNO_3$ PERMEABILITY. AVERAGE OF 3 DETERMINATIONS

(Hibernal and Hyslop average of 2)

| Group (increasing hardiness) | Variety | O.P., atm. | Perm., mols per hr. |
|---|---|---|---|
| 1 | Milwaukee | 28.7 | 0.23 |
| | Delicious | 28.0 | .31 |
| 2 | Alexander | 32.3 | 0.33 |
| | Fameuse | 25.2 | .37 |
| | McIntosh | 29.4 | .30 |
| | Wealthy | 25.9 | .42 |
| 3 | Wolf River | 36.8 | 0.43 |
| | Patten Gr. | 27.3 | .53 |
| 4 | Hibernal | 36.1 | 0.49 |
| | Hyslop | 28.0 | .66 |

### 3. *Permeability to Polar Non-electrolytes*

Two extreme types of non-electrolytes were tried: (1) relatively polar compounds with small molecules which, according to the lipoid-sieve theory of the plasma membrane, pass like water and electrolytes through the pores, and (2) apolar compounds with large molecules which are lipoid-soluble and pass through the substance of the membrane.

As examples of the former, thiourea, urea or glycol (molecular weights 76, 60 and 62, respectively) were used as was found convenient, depending on the permeability of the material. Although hardiness is accompanied by an

increase in osmotic pressure of only about 20% (Levitt and Scarth (9)), permeability to thiourea and urea rises 300%. On the other hand, those species incapable of hardening (*i.e.*, sunflower, castor bean) exhibited no

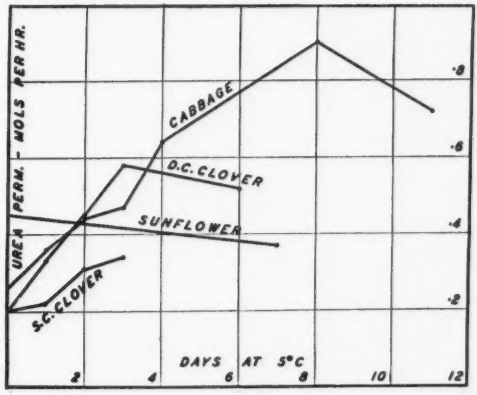


FIG. 3. *Effect of "hardening" temperature on the permeability of hardy (cabbage and clover) and tender (sunflower) herbaceous plants to urea (clover) and thiourea (cabbage and sunflower).*

change either in osmotic pressure or in permeability, on exposure to hardening temperatures. The same change occurs no matter what particular polar substance is used (Table X).

TABLE X

PERMEABILITY OF HARDENED AND UNHARDENED CABBAGE SEEDLINGS (CHLORENCHYMA CELLS) TO THIOUREA AND GLYCOL

| | O.P., atm. | Deplas., min. | | Perm., mols per hr. | |
|---|---|---|---|---|---|
| | | Thiourea, 0.75 *M* | Glycol, 0.75 *M* | Thiourea, 0.75 *M* | Glycol, 0.75 *M* |
| Unhardened | 10.6 | 60 | 13 | 0.52 | 2.40 |
| Hardened | 13.6 | 15 | 3½ | 1.16 | 5.00 |

Twigs of woody plants in their winter state were tested for urea permeability before and after being kept in the laboratory for a number of days. Table XI shows that there is a decrease in all cases. The final reading ranged from 1/2 in *Picea* to 1/11 in *Catalpa*, of the original. The greater and more rapid the decrease, the earlier did the buds open. This suggests a possible relation of permeability to dormancy, but a study of seasonal changes does not educe a very good correlation in this regard.

*Seasonal changes in urea permeability.* As with potassium nitrate, tests were made throughout the year. Here again the minima always occurred when the plant was tenderest to frost (May-July) and the maxima when most resistant (Fig. 4). The extremely high winter values for *Liriodendron* and

TABLE XI

CHANGE IN OSMOTIC PRESSURE AND PERMEABILITY OF TWIGS KEPT AT ROOM TEMPERATURE

| Species | Date | Days at room temp. | O.P., atm. | Conc. urea, mols | Deplas. time, min. | Molar penetration per hour |
|---|---|---|---|---|---|---|
| *Hydrangea paniculata* | Feb. 23/34 | 0 | 27.3 | 1.86 | 30 | 1.01 |
| | | 4 | 18.6 | 1.4 | 90 | .39 |
| | | 7 | 14.9 | .93 | 60 | .40 |
| | | 11 | 14.9 | .93 | 55 | .44 |
| *Caragana arborescens* | Mar. 14 | 0 | 30.2 | 2.79 | 40 | 1.15 |
| | | 15 | 14.3 | .93 | 145 | .19 |
| *Picea pungens* | Mar. 16 | 0 | 21.2 | 2.79 | 120 | .52 |
| | | 11 | 18.0 | .93 | 30 | .46 |
| | | 31 | 13.6 | .93 | 125 | .24 |
| *Pterocarya rhoifolia* | Mar. 23 | 0 | 36.1 | 2.79 | 30 | 1.20 |
| | | 21 | 19.9 | 1.86 | 40 | 1.16 |
| *Catalpa hybrida* | April 11 | 0 | 28.0 | 2.79 | 16 | 3.12 |
| | | 5 | 14.9 | .93 | 64 | .38 |
| | | 12 | 13.0 | .93 | 111 | .29 |
| *Aesculus hippocastanum* | April 19 | 0 | 21.9 | 2.79 | 21 | 2.93 |
| | | 29 | 12.4 | .93 | 86 | .41 |

*Catalpa* are not comparable to the others, for they were based on measurements of "protoplasmal deplasmolysis". In other words, an ultra rapid penetration of the outer membrane occurred far in advance of the penetration through the tonoplast. In some cases there is another maximum during August, but, as we shall see later, drought resistance is also correlated with urea permeability, and this may be the explanation. By September, all the values have risen considerably from the spring minimum, whereas in the case of potassium nitrate permeability the initial rise occurs about November. Furthermore, the first drop in permeability to potassium nitrate is registered at the end of March—a full month ahead of the decrease in urea permeability.

As for varietal resistance, the results are not so clear-cut as with potassium nitrate permeability. However, during the winter months the two hardy apple varieties do show a higher permeability than the more tender ones. Since increased urea permeability apparently parallels the early stages of hardening, it is not surprising to find it less strikingly correlated with varietal hardiness than potassium nitrate permeability.

Assuming that the change in rate of penetration is due to alterations in the pore size of the plasma membrane, then it appears that the initial increase in diameter allows more rapid entrance of urea but not of potassium nitrate. Any further enlargement enables potassium nitrate to penetrate more readily. The summer drought was therefore sufficient to speed up urea permeability, but only when full pore size was attained (during winter) did potassium nitrate permeability increase. Furthermore, as soon as the height of the

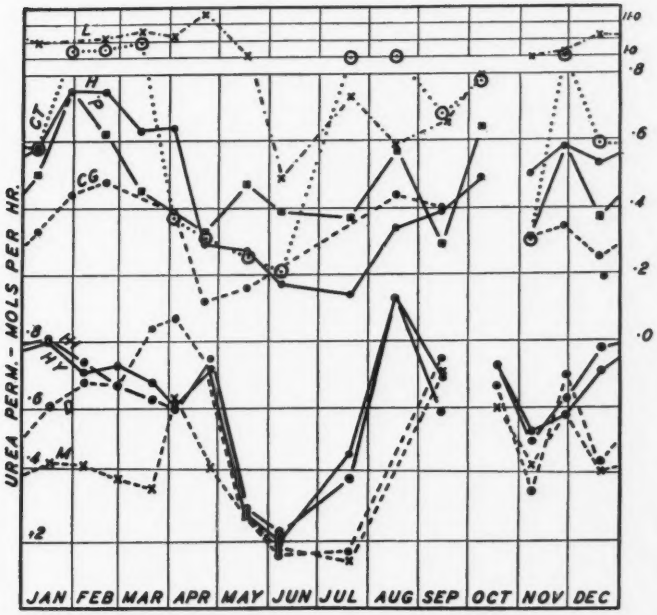


FIG. 4. *Seasonal changes in urea permeability of hardy woody plants, determined on cortical cells of current growth. Oct. 1934–Oct. 1935.*

*Upper Curves*

*CG* = *Caragana arborescens*
*L* = *Liriodendron tulipifera*
*CA* = *Catalpa hybrida*
*H* = *Hydrangea paniculata*
*P* = *Picea pungens*

*Lower Curves (apple varieties)*

*HI* = *Hibernal* } *Hardy*
*HY* = *Hyslop* }

*M* = *Milwaukee* } *Less hardy*
*D* = *Delicious* } *than above*

winter began to recede, the decrease in pore size was mirrored in a reduced potassium nitrate penetration rate, but only later were the pores sufficiently reduced to cause a drop in urea permeability.

4. *Permeability to Apolar Substances*

From the list of substances used by Collander and Bärlund (2), urethane coming at the top of their list and succinimide some way down the series were chosen. Only comparative permeability could be tested on account of their quick penetration, which necessitated using the technique of finding the lowest concentration causing plasmolysis. Also, as these concentrations of the pure substance were high and toxic, mixed solutions of the test substance and of calcium chloride having a partial osmotic pressure equal to that of the cell sap had to serve the purpose. Table XII gives the results of an experiment in which hardening, while it induced osmotic pressure to increase 32% and thiourea permeability 100%, affected the penetration rate of urethane and succinimide not at all.

An attempt was also made to determine the permeability of hardy and non-hardy cells to dextrose. Since this substance penetrates cells very slowly, if at all, the method adopted was such as to detect even very slight penetration. Sections of hardened and dehardened *Catalpa* were placed in isotonic solutions of dextrose, so that about half the cells showed incipient plasmolysis. These cells were then examined for deplasmolysis from time to time, but neither hardened nor dehardened cells showed any change even after 24 hours. At the end of 48 hours those cells still alive had deplasmolyzed, but this was obviously due to injury.

TABLE XII

PERMEABILITY OF HARDENED AND UNHARDENED CABBAGE SEEDLINGS (CHLORENCHYMA CELLS) TO POLAR AND APOLAR SUBSTANCES

| | Unhardened | Hardened |
|---|---|---|
| O.P. | 10.6 atm. | 14.3 |
| Thiourea perm. | .82 *M* per hr. | 1.64 |
| **PARTIAL CONCENTRATION CAUSING INCIPIENT PLASMOLYSIS** | | |
| Urethane | .50 *M* | .50 *M* |
| Succinimide | .05 *M* | .05 *M* |

Collating our results with electrolytes, polar non-electrolytes and apolar non-electrolytes respectively, we find that the maximum effect of hardening on permeability is greater the more polar the compound, and no effect is shown with apolar or lipoid-soluble substances. We note also that the seasonal rise in permeability appears first in the case of urea, etc., and much later in the case of salt. The seasonal fall in spring occurs in the reverse order. If the permeability change is due to change in pore diameter, the inference is that potassium nitrate requires wider pores than urea.

*Relation of permeability to osmotic pressure.* Most of our records show a parallel variation of osmotic pressure and permeability, but there is also plenty of evidence to prove that the connection is not a necessary one. For example, the osmotic pressure of *Helianthus* was found to increase 80% from an early stage of growth to the flowering period, while permeability remained unchanged (Table XIII).

*Helianthus* is incapable of hardening, but an experiment with cabbage, in which cut leaves were allowed to

TABLE XIII

OSMOTIC PRESSURE AND PERMEABILITY OF *Helianthus* AT DIFFERENT STAGES OF GROWTH

| Age, days | O.P., atm. | Deplasmolysis, min. in .75 *M* thiourea | Permeability, mols per hr. |
|---|---|---|---|
| 54 | 10.6 | 70 | 0.45 |
| 80 | 12.4 | 50 | .46 |
| 89 | 14.3 | 32 | .45 |

TABLE XIV

EFFECT OF SUGAR FEEDING ON OSMOTIC PRESSURE AND PERMEABILITY OF CABBAGE LEAVES (CHLORENCHYMA CELLS)

(Average of 3 plants)

| Days in $\frac{M}{5}$ dextrose | O.P., atm. | Conc. thiourea, mols | Deplas., min. | Permeability, mols per hr. |
|---|---|---|---|---|
| 0 | 10.9 | 0.75 | 42 | 0.71 |
| 2 | 13.0 | 1.00 | 60 | .60 |

take up dextrose solution until the osmotic pressure of the chlorenchyma cells rose 20% also gave no increase but rather a slight decrease of permeability as a result (Table XIV).

Increase of frost resistance in such a case is slight, being merely proportional to the depression of the freezing point and not comparable to the increase which is associated with an equal osmotic change combined with a rise in permeability as produced by normal hardening. These experiments were confined to urea permeability, but we have seen with regard to electrolyte permeability that its seasonal rise and fall does not synchronize with change in osmotic pressure.

## Relation of Permeability to Hardiness Induced Otherwise Than by Cold

*Wilting.* Cold resistance as well as drought resistance of plants is increased by restriction of their water supply. It is interesting to know whether the development of drought resistance in a typical xerophyte is attended by any permeability change. *Spartium junceum,* a switch type of xerophyte, was kept unwatered for two weeks. During the second week, wilting began and progresssed greatly, osmotic pressure rose 35%, and permeability was almost doubled (Table XV).

TABLE XV

OSMOTIC PRESSURE AND PERMEABILITY CHANGE DURING INCREASE OF DROUGHT RESISTANCE OF *Spartium*

| Days unwatered | O.P., atm. | Deplas., min. | Conc. urea, mols | Urea perm., mols per hr. |
|---|---|---|---|---|
| 0 | 18.0 | 66 | 1 | 0.27 |
| 7 | 17.4 | 55 | 1 | .37 |
| 14 | 24.6 | 60 | 2 | .66 |

*Checking.* Other conditions besides drought which cause a check in growth are known also to increase hardiness, both to drought and to cold.

Two sets of cabbage seedlings grown side by side afforded a comparison in this respect, in which permeability was also tested. One set somewhat older than the other had been severely checked in the early stages of growth by a heavy aphid infestation combined with very dull weather, and as a result showed a different morphology and much greater resistance to wilting than the younger set. The osmotic pressure of the unchecked was 10.6 atm. and of the checked 12.1 atm. The permeability of the unchecked was 0.42 and of the checked 0.86 mols per hour, a conspicuous increase with checking (Table XVI).

TABLE XVI

OSMOTIC PRESSURE AND PERMEABILITY OF CHECKED AND UNCHECKED CABBAGE SEEDLINGS (CHLORENCHYMA CELLS)

| | O.P., atm. | Deplasmolysis, min. in .75 *M* thiourea | Permeability, mols per hr. |
|---|---|---|---|
| Unchecked | 10.6 | 71 | 0.42 |
| Checked | 12.1 | 28 | .86 |

*Low nitrogen supply.* Cabbage seedlings were grown in sand cultures and watered every two weeks with Knop's solution or a modification of it where low amounts of nitrogen were required. In the latter cases, calcium chloride was added to replace the calcium nitrate and potassium sulphate instead of potassium nitrate. Whether by checking growth or in some more direct fashion, low nitrogen supply increases hardiness. That it also increases permeability is shown in Table XVII.

In short, by whatever means cold resistance is induced, it is accompanied by an increase of cell permeability to polar compounds.

TABLE XVII

EFFECT OF QUANTITY OF AVAILABLE NITROGEN ON COLD RESISTANCE, OSMOTIC PRESSURE AND PERMEABILITY OF CABBAGE PLANTS

| N, p.p.m. | % injury* (average of 9 plants) | O.P., atm. | Permeability,* mols per hr. (average of 5 plants) |
|---|---|---|---|
| 10 | 25 | 11.8 | 0.55 |
| 25 | 15 | 11.2 | .54 |
| 50 | 40 | 10.6 | .57 |
| 100 | 90 | 10.0 | .45 |
| 200 | 80 | 9.4 | .32 |
| 300 | 85 | 10.6 | .37 |

**Injury tested on one set of 9 plants.*
*Permeability tested on another set of 5 plants.*

## Discussion

The principal conclusion from this research is that cell permeability is discovered to be correlated with hardening against frost, and indeed to show a greater and probably a more strictly parallel variation with hardiness than any of those factors to which cold resistance has hitherto been ascribed.

Hardiness and permeability vary together in the life of a plant not only in the normal seasonal rhythm and in relation to temperature change, natural or artificial, but also in response to various other factors, such as water supply, nutrition and even disease. Osmotic pressure and permeability, while frequently linked, may also vary independently, in which case frost resistance follows permeability more closely.

The degree of change in permeability (in relation to hardening) varies greatly with the type of penetrant and also with the type of plant. As regards the former, the rule seems to be that the change is greater the more polar the substance, being widest with the strong electrolyte used (potassium nitrate) and disappearing altogether with the least polar compounds (urethane, etc.). As regards differences in plant species, the permeability change depends upon their powers of cold resistance. Thus the following classification may be made, correlating hardiness and permeability.

*Non-hardy*—No increase in permeability on exposure to low temperature.

*Semi-hardy*—Permeability to water and polar non-electrolytes with small molecules (urea, etc.) increases on exposure to low temperature and to other conditions causing hardening.

*Hardy*—Permeability to water and polar non-electrolytes increases as in semi-hardy plants but to a much greater degree; also permeability to strong electrolytes (potassium nitrate) is measurable in the unhardened state and greatly increased on hardening.

It seems likely that the permeability test may prove to be of practical use in predicting hardiness, especially in the case of woody plants, the resistance of which is brought to the test only in exceptionally severe winters. The permeability, especially to potassium nitrate, of the cells of these plants in the winter condition, affords an easily applied measure of their potential frost resistance without the necessity of waiting for a test season. At any rate, our results demonstrate that the relative hardiness of the apple varieties tested can be judged by this means, and in breeding new types it is merely comparison within species that is required.

The degree of permeability change which our measurements record is probably unparalleled in the literature for normal non-pathological conditions. The mechanism underlying it is therefore a problem of general interest, as well as in relation to the study of hardiness. It is evident that the type of permeability which is here measured is the so-called passive or physical permeability. It obeys Fick's law of diffusion, and the temperature effect is alike on hardened and unhardened cells. The temperature coefficient is certainly high (2.1), but this applies also to other cases of passive permeability. If the permeability measured is of this type, its increase on hardening is to be ascribed to a change in the properties of the protoplasmic membrane or membranes, and, from the fact that only polar or non-lipoid-soluble substances are affected, that change is inferred to be a widening of its aqueous "pores".

As will be described in a subsequent paper, another physical change, *viz.*, a lowering of viscosity, either in the protoplasm as a whole or at least in its surface layer, also attends hardening.

Now, it is well known that salts of the alkali metals tend to produce both these effects, *viz.*, increase of permeability and liquefaction of the protoplasm. Furthermore, calcium ions which antagonize the action of these salts also tend to neutralize the effects when they result from hardening. The inference on both counts is that the underlying colloidal change associated with hardening is similar to that induced by salts of the alkalis. The nature of this change in protoplasm is suggested by physical analogy. In the case of those hydrophilic colloids which display antagonism of ions, the action of the alkalis is toward increased dispersion and hydration of the particles. This, then, seems at present the most reasonable hypothesis of the permeability increase, namely a looser packing of the organic micellae or molecules and an enlargement of the aqueous phase of the plasma membrane. The evidence is not decisive as to whether this change extends to the protoplasm as a whole. This will be discussed in a later paper.

We come now to the question of what role, if any, the permeability change plays in determining resistance to frost. The close correlation of permeability

with hardiness, when induced by the most varied factors and in most varied types of plant, inclines to the view that the correlation is more than incidental. The exact significance of the relation, however, cannot be understood unless we know precisely the mechanism of frost injury.

On the theory that over-concentration of cell solutes may be the injurious factor, escape of the more toxic ingredients of the sap might tend to obviate this (Lidforss). In spite of the high permeability of hardened cortical cells of trees to potassium nitrate as measured plasmolytically, there is no evidence of exosmosis of salts or other substance from the cells under natural conditions. Mr. J. L. Howatt, working in this laboratory, tried immersing cortical tissue of twigs in winter condition in distilled water for periods up to three days. Chemical tests applied to the extract gave only a faint unidentifiable precipitate with lead acetate. There was no evidence of appreciable leaching. It is true that in winter, substances pass from storage cells into the tracheal sap, but these are largely sugars to which we find no evidence of increased permeability as tested plasmolytically, and which are probably "secreted" by a more complex process than simple diffusion.

The very basis of the toxic concentration theory is attacked by Iljin and others on the grounds that an equal concentration of the cell sap by plasmolysis has no ill effect. While this does not eliminate the possibility that the time factor in frost action may be connected with toxic concentration, it is sufficient proof that immediate frost injury must be due to some other cause.

As will be shown in another paper, the evidence is in favor of a mechanical theory of frost injury, and certain types of mechanical injury have been observed.

*Quick freezing.* As regards the type of mechanical injury which results from quick freezing, the part which permeability must play is not hard to understand. The damage in this case is caused by ice forming inside the cells, and this only happens if the cell sap is cooled faster than its freezing point is lowered by concentration of its sap. The sap tends to be concentrated, of course, because crystallization always begins outside of the cells and the crystals grow by drawing water from the cells. The limiting factor is the rate at which water can escape from the cells. Hence, the higher the permeability to water, the less the danger of intracellular freezing and of death. In nature, very rapid freezing may occur momentarily after super-cooling; hence such protection is needed.

*Slow freezing.* There is ample evidence that on occasion damage is produced by the development of large ice masses which press upon the cells and push them apart. The smaller the crystals the less is the likelihood of mechanical injury of this type. Large crystals are the result of slow crystallization, and if the rate of crystallization is limited by the rate at which water can exosmose from the cells, a higher rate of permeability to water would tend to reduce crystal size and hence minimize mechanical damage of this

type. This may explain why large ice masses are not formed in hardy plants, and indeed it may explain *how* they are hardy. In both quick and slow freezing, the small cell size which is characteristic of hardy plants would also be useful in aiding the rapid exit of water.

Further discussion is reserved until other physical changes in the protoplasm have been described.

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# ROOT ROTS OF THE RASPBERRY[1]

By G. H. Berkeley[2]

## Abstract

The investigations reported here are concerned with isolations from diseased raspberry roots, preliminary inoculations with isolates, and microscopic examination of naturally and artificially infected roots.

The following fungi were isolated from naturally infected roots: *Coniothyrium Fuckelii, Cylindrocarpon radicicola, Fusarium* sp., possibly *F. orthoceras, Cylindrocladium* sp., *Pythium* spp., *Rhizoctonia Solani, Rhizoctonia* sp. (orchid type), and in preliminary inoculation experiments each fungus was found to be capable of producing necrotic lesions on healthy roots. Microscopic examinations of roots artificially inoculated with pure cultures of the above fungi, showed in the roots the presence of the fungus used for inoculation. In addition the "phycomycetous mycorrhizal" fungus already associated with root rot of strawberries and tobacco was observed to be almost always present in roots of affected raspberry plants, and, to a lesser extent, in apparently healthy roots from apparently normal plants. Nematodes, especially *Anguillulina pratensis*, the meadow nematode, were present in and on roots from certain soils, while they were absent from roots from other soils. Strawberry and raspberry seeds were sown in sterilized and non-sterilized affected soil with the result that the roots in sterilized soil appeared to be healthy, while those in the non-sterilized soil became affected with necrotic lesions.

Evidence is given which shows not only that certain symptoms of raspberry root rot are similar to the symptoms of strawberry root rot, which is considered to be a major factor in the degeneration of strawberries in both Europe and America, but also that many of the fungi and nematodes generally conceded to be associated with root rots of strawberry are likewise associated with root rots of raspberry. In the Fraser Valley, British Columbia, where certain raspberry plantations appear to be heavily infected with root rots, the possibility that these root rots may play an important role in connection with the unthriftiness of such plantations should not be overlooked.

## Introduction

Macroscopic and microscopic examination of raspberry roots from certain districts in Ontario and British Columbia has shown the presence of numerous necrotic lesions on otherwise apparently healthy roots, and within such lesions, fungi and nematodes were commonly found in quantity. In Ontario the degree of infection observed to date is slight, and apparently has had little, if any, effect on growth. In raspberry material received from the Fraser Valley, British Columbia, the percentage of diseased roots was much higher. In fact in several plantations where raspberries had failed, roots were examined on which not a single healthy rootlet could be found. Though there appeared to be a direct correlation between the percentage of diseased roots and the general lack of vigor in such plants as expressed by pronounced dwarfing of canes accompanied by curling, bronzing and scorching of the leaves, and death of individual canes or the entire stool, the rotting of the roots may not be entirely responsible for the stunting or death of above-ground parts. The possibility that mineral deficiencies, unbalanced soil nutrients or toxins may predispose raspberry roots to parasitic attack must also be considered.

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Nevertheless, where a large percentage of the roots of raspberry plants are infected with parasitic fungi and nematodes, it is believed that they play an important role in this connection.

## Literature

So far as the writer is aware, there is no reference in the literature to raspberries being attacked by a complex root rot, with which several different organisms may be associated. On the other hand, root rots of this nature have been reported on cereals, sugar cane, pineapples, flax, peas, tobacco, strawberries and other crops. Cereals are subject to several root rots which are generally recognized today as beng one of the major factors associated with losses in these crops. For example, browning root rot caused by *Pythium* spp. (32, 33, 34) which, according to Vanterpool and Ledingham (32) is widespread over Saskatchewan and occasions severe losses in some seasons; take-all, caused by *Ophiobolus graminis* Sacc. (10, 23, 25, 27), of which Russell (23) states that "under conditions favorable to this disease losses of 25 to 75 per cent of the crop have not been uncommon in parts of Australia, Union of South Africa, the U.S.A., and Canada"; and foot rots, caused by *Helminthosporium sativum* and *Fusarium culmorum* (6, 7, 13, 26). Pineapples in Hawaii, and sugar cane in Hawaii, the Philippines and the United States are subject to root rots caused by *Pythium* spp. (8, 9, 28). Root rots of flax (5) in the United States have been assigned to *Fusarium lini*, *Asterocystis radicis*, *Thielavia basicola*, *Pythium megalacanthum* and *Rhizoctonia* spp. Peas are affected with a serious root rot caused by *Fusarium Martii* var. *Pisi*, and *Aphanomyces euteiches*. Tobacco is subject to two distinct root rots, black root rot caused by *Thielaviopsis basicola*, and brown root rot, the cause of which is not definitely established. Recently Koch (20) has shown that several different fungi are associated with *Thielaviopsis basicola* in black root rot of tobacco. Strawberries in Europe are attacked by two types of root rot, the red core (1), and black lesion types (2). In Canada, U.S.A., Australia and Africa the black lesion type is common. Strong and Strong (29) have presented evidence to the effect that *Hainesia Lythri* (Desm.) v. Höhn., and *Coniothyrium Fuckelii* Sacc., cause a black lesion type of root rot in Michigan which is responsible for unthriftiness and patchiness in strawberry plantations, often resulting in losses ranging from 5% to as high as 60%. Berkeley and Lauder-Thomson (2) found these same two fungi and, in addition, *Fusarium orthoceras* App. and Woll., *Cylindrocarpon radicicola* Wr., and *Pachybasium candidum* Sacc., to be capable of affecting the roots of strawberry in England, thereby causing stunting and often death of affected plants. In Ontario, Walker (35) found that species of *Ramularia* were associated with root rot of strawberries. Truscott (30) has reported that species of *Pythium*, *Alternaria*, *Verticillium*, *Cylindrocladium*, *Rhizoctonia* and *Asterocystis* and a "phycomycetos mycorrhizal" fungus are also capable of parasitizing the roots of strawberry. In a microscopical study, Hildebrand (18) observed that nematodes as well as the fungi mentioned above, were generally present in the roots of

affected strawberries. Berkeley (3) describing the effects of root rot under Ontario conditions states that "individual plantations have been so attacked that what appeared to be a promising plantation in May and June was later in the season ploughed under as unprofitable". In Oregon, Zeller (36) reports a root rot of strawberry caused by *Rhizoctonia Solani* which is one of the chief factors causing variability and unevenness in Marshall strawberry plantings in the Pacific northwest.

The above citations and statements are sufficient to show that root rots are considered to be of considerable importance in the culture of various crops. Whether or not root rot will be found to be equally important in connection with raspberry culture has yet to be ascertained.

## Material and Methods

The results recorded in this paper are based on (1) a small number of diseased roots observed in Ontario, and (2) root material from British Columbia collected between July and September 1935 from three farms designated for convenience as Farms P, H, and D. Also transplants were grown outdoors in pots in sterilized and non-sterilized soil from each of the three farms mentioned. In addition, strawberries and raspberries were grown from seed in sterilized and non-sterilized naturally infected soil.

Root material was prepared for microscopic examination by boiling for one minute in lacto-phenol and acid fuchsin. After boiling, the stained material was mounted in clear lacto-phenol and gently crushed under the cover slip. Inoculations were effected by the preliminary method described by Berkeley and Lauder-Thomson (2) for strawberries.

## Experimental

### Preliminary Tests

As a preliminary test to ascertain the possibility of parasitic attack on raspberry roots, clonal raspberry suckers growing in soil which produced normal plants were transplanted into sterilized and non-sterilized soil from Farms P, H, and D. By the end of the growing season, when some of these plants were carefully removed from the soil and washed, it was observed that the root system in the non-sterilized soil from each of the three farms was much reduced in size as compared with the roots from the same soil, sterilized (Plate II, Figs. 1 and 2). This reduction in size was largely due to the scarcity of fibrous roots. A macroscopic examination of the newer fibrous roots showed the presence of distinct necrotic lesions, especially on the roots of plants grown in non-sterilized soil. The differences in above-ground growth were equally striking, since strong, vigorous growth occurred in all sterilized soil, but exceedingly poor growth resulted in the non-sterilized soil (Plate II, Fig. 1). In this connection, it should be noted that the sterilization of normally productive soil also brought about an improvement in growth, though the difference was not so pronounced as it was in the case of the unproductive soils.

*Microscopic Examination of Roots*

(a) *From non-sterilized soil.* Microscopic examination of prepared rootlets showed the presence of nematodes and various fungi in the necrotic lesions. The "mycorrhizal" fungus so commonly found in strawberry roots was equally common in affected raspberry roots. In the case of plants from Farms H and D, nematodes were present in and on root lesions in great abundance, while in those from soil from Farm P, nematodes were rarely observed. *Rhizoctonia* (orchid type), *Rhizoctonia Solani*, *Pythium* sp., *Asterocystis* and unidentified mycelium were quite commonly observed in affected tissue (Plates III and IV).

(b) *From sterilized soil.* Though the fibrous roots of plants grown in sterilized soil appeared to be healthy, nevertheless the "mycorrhizal" fungus was observed to be present in roots from soils from Farms P, H and D, as also were a few nematodes in the roots from soils from Farms H and D. The explanation would appear to be that the sterilized soil had become contaminated either by means of the roots of the transplants, or because the pots were sunk in the ground outdoors. In any case, parasitic action was decidedly lessened, as indicated by the greater extent of the root system in sterilized soil, as compared to non-sterilized soil. In the case of the non-sterilized soil, it is assumed that fungus invasion had been progressing throughout the season, resulting in a greatly depleted root system, whereas in the case of the sterilized soil the roots must have been free from parasitic attack during most of the season in order to produce such a large and healthy root system. A microscopic examination of these roots supports this contention, in that fungi were comparatively scarce in the roots from sterilized soil as compared with those from non-sterilized soil; nematodes (Farms H and D) were much fewer in number, and the roots that were attacked appeared intact, whereas similar roots from non-sterilized soil were necrotic.

*Seedlings Grown in Sterilized and Non-sterilized Soil*

To test further the parasitic nature of this suspected root-rot complex of raspberries, seed of both raspberries and strawberries was sown in sterilized and non-sterilized soil from Farms P and H. When the seedlings showed the first true leaf they were carefully washed and examined microscopically. Distinct lesions could be seen with the naked eye on the roots of both strawberry and raspberry from non-sterilized soil, whereas no lesions were apparent on the roots from sterilized soil. When examined microscopically, the presence of the "phycomycetous mycorrhizal" fungus, *Rhizoctonia* (orchid type) and mycelium of unidentified fungi were observed in roots from the non-sterilized soil, while no fungi were observed in the roots from the sterilized soil. Also nematodes were numerous in lesions on the roots from non-sterilized soil from Farm H, though none was observed in roots from non-sterilized soil from Farm P. No nematodes were observed in roots from sterilized soil from either Farm H or Farm P.

The raspberry seedlings in the non-sterilized soil from Farms P and H were considerably stunted as compared with seedlings in the same soils sterilized

In the non-sterilized soil from Farm H the seedlings were only $\frac{1}{4}$ inch high as compared with $\frac{3}{4}$ inch in the same soil sterilized. In the non-sterilized soil from Farm P the seedlings were $\frac{3}{4}$ to 1 inch high as compared with seedlings $1\frac{3}{4}$ to 2 inches high in the same soil sterilized. The fact that germination was low and growth poor in the soil from Farm H, even after it had been sterilized, suggests that it may be deficient in certain elements, or that it may contain a toxin or toxins.

In evaluating the above differences in growth, the beneficial effects of sterilization on the physical and chemical nature of the soil as opposed to the effect on its biology, should not be overlooked.

These tests strongly suggest a root-rot complex caused by fungi and nematodes capable of attacking either strawberries or raspberries.

## Isolations from Naturally Affected Roots, Inoculations with Isolates, and Microscopic Examination of Inoculated Roots

Representatives of nine genera of fungi were isolated from lesions on diseased roots, and inoculation tests with these isolates have demonstrated that members of six genera were capable of parasitizing raspberry roots. These will be taken up separately. Microscopic examination of the inoculated roots showed the presence of the fungus used as inoculum. Also microscopic examination of naturally diseased roots showed the presence in affected root tissue of the "phycomycetous mycorrhizal" fungus and nematodes.

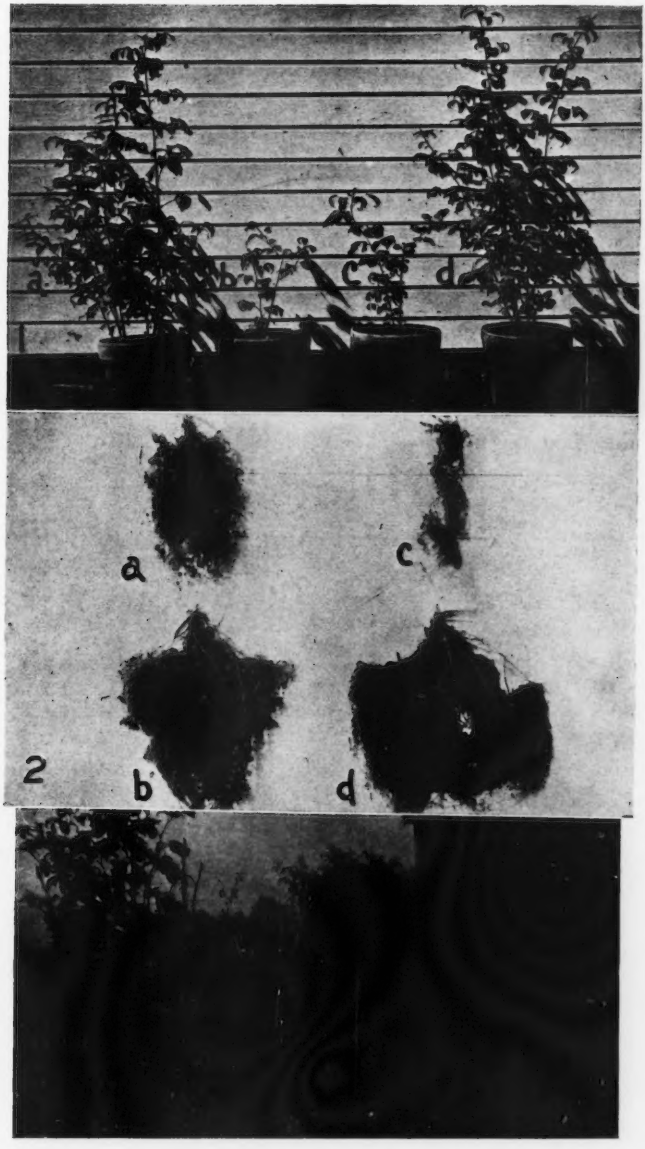
### (a) *Coniothyrium Fuckelii* Sacc.

Inoculation with a strain of *Coniothyrium Fuckelii* Sacc., isolated from roots and crown of affected raspberry plants produced brown necrotic lesions within 4 to 5 days. Microscopic examination of such lesions showed the presence of abundant mycelium, whereas the healthy areas of roots on both sides of the lesion showed no mycelium. Check roots were healthy. The strain of *Coniothyrium Fuckelii* isolated from strawberry roots was also found to be capable of parasitizing raspberry roots. Berkeley and Lauder-Thomson (2) have shown that *Coniothyrium Fuckelii* isolated from raspberry canes was capable of attacking roots of strawberry.

### (b) *Cylindrocladium* sp.

A species of this genus was isolated, which appears to be similar to *C. scoparium* Morgan, isolated from strawberries in Ontario by Truscott (30) who found it to be a virulent parasite of strawberry. Preliminary inoculation tests demonstrated that the species isolated from raspberry not only was a strong parasite on raspberry but also was equally virulent on strawberry roots. Microscopic examination of roots following artificial inoculation showed the presence of the typical mycelium and sclerotia of the *Cylindrocladium* sp., within the tissue of the roots. This evidence, therefore, suggests that *Cylindrocladium* sp., must be considered a parasite of raspberry roots (Plate III, Figs. 5 and 6).

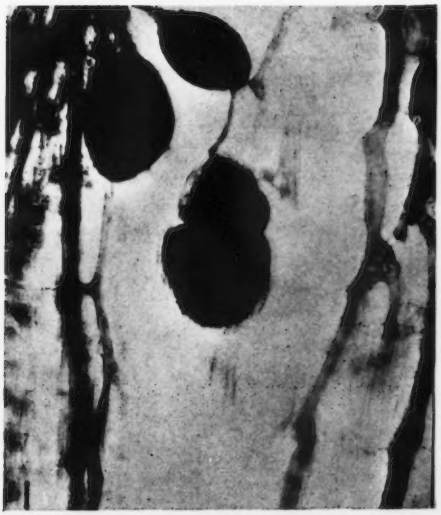
*Strawberry and raspberry plantations, British Columbia. 1: Strawberry root rot in foreground. Note that many plants have died. 2: Raspberry root rot on Farm P. Note bulk of plantation has died. 3: Vigorous raspberry plants on Farm P before the "dying out" had become as severe as shown in 2.*

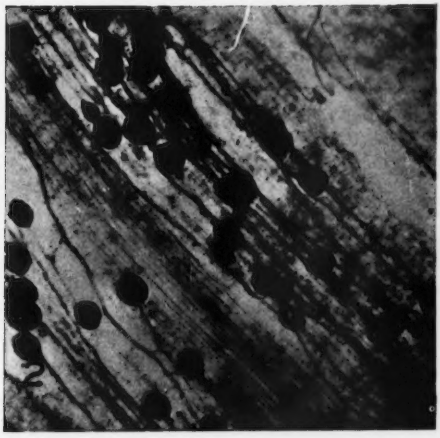



*Raspberry root rot. 1: Raspberry plants grown in sterilized and non-sterilized infected soil; (a) sterilized soil from Farm H; (b) non-sterilized soil from Farm H; (c) non-sterilized soil from Farm P, and (d) sterilized soil from Farm P. 2: The roots from plants illustrated in 1: (a) roots from non-sterilized soil from Farm P; (b) roots from sterilized soil from Farm P; (c) roots from non-sterilized soil from Farm H, and (d) roots from sterilized soil from Farm H. 3: Two dead plants which had made good growth the previous year. This is the type of decline as found on Farm P.*

*Photomicrographs. 1: Asterocystis in root tissue. 2: Spores of Pythium sp. in root tissue as result of inoculation, × 365. 3: Rhizoctonia sp. (orchid type) showing the monilioid cells in naturally affected roots, × 365. Similar cells were also observed in inoculated roots. 4: Nematodes in and on necrotic root tissue, × 365. 5: Cylindrocladium sp. Mycelium in root tissue as a result of inoculation, × 365. 6: Sclerotial-like bodies of Cylindrocladium sp., on root tissue as result of inoculation, × 365. Both the mycelium and sclerotial body were similar to those found in cultures of the inoculum.*

PLATE IV





*"Phycomycetous mycorrhizal" fungus. Photomicrographs. 1: Mycelium and vesicles of "mycorrhizal" fungus, × 365. 2: Same as in 1, × 77. 3: Mycelium of "mycorrhizal" fungus. Note irregular coarse mycelium with peculiar branching and some septation. 4: Photograph of two rootlets showing necrotic lesions caused by parasitic organisms.*

(c) *Cylindrocarpon radicicola* Wr.

Berkeley and Lauder-Thomson (2) and Truscott (30) have demonstrated that a species of *Cylindrocarpon* is capable of parasitizing the roots of the strawberry. Van Hell (31) has proved that *C. radicicola* Wr. is capable of causing a root rot of lily and narcissus bulbs.

Three isolates of *C. radicicola* have been obtained from diseased raspberry roots, each of which has proved to be parasitic when tested. Microscopic examination of inoculated roots has shown the presence of mycelium and chlamydospores identical with those found in cultures of the isolates.

(d) *Species of Fusarium*

Several different members of this genus have been isolated, but one species which appears to be similar to *F. orthoceras* App. and Woll. as reported on strawberries by Berkeley and Lauder-Thomson (2) has been isolated on several occasions and has been proved by inoculation tests to be parasitic on raspberry rootlets. Microscopic examination of inoculated roots has shown the presence of mycelium and chlamydospores similar to those found in the culture of the fungus used as inoculum.

(e) *Species of Rhizoctonia*

*Rhizoctonia* spp., of both the Solani and orchid types have been isolated from affected raspberry roots. Microscopic examination of naturally affected roots has also shown the presence of these two types within the root tissue. Truscott (30), and Hildebrand (18) have recently pointed out that *Rhizoctonia* (orchid type) is found in diseased strawberry roots in Ontario, and Zeller (36) considers *Rhizoctonia Solani* Kühn, as the cause of a strawberry root rot in Oregon. Both *Rhizoctonia Solani* and a form of the orchid type, similar to, if not identical with that described by Truscott (30) and Hildebrand (18) on strawberry, have produced dead roots or distinct brown lesions on raspberry roots within 4 to 5 days after inoculation. Microscopic examination of roots artificially inoculated with *Rhizoctonia* (orchid type) has shown the presence within the affected tissue of the characteristic monilioid cells (Plate III, Fig. 3), while artificial inoculations with *R. Solani* have shown the sclerotial groups and mycelium typical of this species. Therefore as a result of this evidence these two species of *Rhizoctonia* must be considered capable of attacking the roots of raspberry.

(f) *Species of Pythium*

Members of this genus were more commonly encountered than were members of any other group. Three morphologically different strains were isolated and inoculations gave positive results with each *Pythium* isolate tested. Microscopic examinations of the artificially inoculated roots showed in each case that the typical mycelium and spores were present in the root tissue. Definite killing of the roots occurred within 3 days from time of inoculation (Plate III, Fig. 2).

(g) *The "Phycomycetous Mycorrhizal" Fungus*

An endophyte of the phycomycetous type of "mycorrhizal" fungus, very similar to the one associated with strawberry roots (18, 21, 30) is the most prominent and conspicuous organism found in raspberry roots. Though no one has as yet been successful in culturing this fungus, evidence is accumulating which points to the fact that this fungus may be a root parasite. Truscott (30) believes that this fungus is one of the more important pathogens of the strawberry and says "The evidence that this fungus is strictly a parasite when growing in the strawberry is indisputable" (Plate IV, Figs. 1-3).

Hildebrand and Koch (19) in a study of the "phycomycetous mycorrhizal" fungus in connection with tobacco and strawberry root rots, state that in some instances necrosis of tissue in tobacco roots could be correlated with the presence of the "mycorrhizal" endophyte, but in the case of strawberry roots, necrosis was seldom observed, though depletion of starch in invaded cells was a common occurrence.

In the naturally affected raspberry roots examined, the mycelium, vesicles and arbuscules of this fungus were almost always present. In some cases the root cells were so filled with this fungus that it is hard to conceive of the possibility of normal root activity under such conditions. Though the "mycorrhizal" fungus was almost invariably present in necrotic lesions of naturally affected raspberry plants, yet many apparently healthy roots contained an abundance of this fungus with no necrosis whatever. A few cases were observed where the "mycorrhizal" fungus was present in and on root hairs.

(h) *The Genus Asterocystis*

In a few diseased roots resting cells of *Asterocystis* were present in some abundance, though little or no necrosis of host tissue was observed. *Asterocystis* is generally considered to be an obligate parasite and therefore the presence of this fungus in root tissue suggests that it may be a factor in the raspberry root-rot complex (Plate III, Fig. 1).

(i) *Nematodes*

Several different nematodes have been found associated with root rots of raspberry in certain districts in British Columbia, but *Anguillulina pratensis* (de Man 1880) Goffart 1929*, was present in greatest numbers. In root material from a farm in the Hatzic area nematodes were present in great numbers both in and on root lesions. In root material from other farms nematodes were almost entirely lacking or but rarely present.

In this connection, however, it is interesting to note that no nematodes were present in roots obtained from the most severe case of degeneration, observed in British Columbia on Farm P, while nematodes, *A. pratensis* included, were unusually abundant in and on root lesions from roots obtained from plantations much less severely affected, for instance at Farm H (Plate

* *Identified by Division of Nematology, Bureau of Plant Industry, U.S. Department of Agriculture.*

III, Fig. 4). Also when both raspberry and strawberry seed was planted in the unproductive soil from the above farms and the resulting seedlings examined with the microscope as soon as the first true leaf was formed, at which time the roots were 1 to 1½ inches long, it was observed that nematodes were abundant in lesions on roots from Farm H soil, while they were lacking in lesions on roots from Farm P soil, results which are in close agreement with the findings obtained by examination of naturally infected roots from both farms.

*A. pratensis* is generally considered to be a root parasite capable of attacking many different plants, however, since it has not been cultured, proof of its pathogenicity on raspberry has not been possible, but the fact that *A. pratensis* in some cases was quite general in necrotic lesions on new rootlets (seedling tests) suggests parasitism, since they were invariably associated with necrotic tissue and were sometimes the first organisms observed in such lesions.

Hastings (16) considers *A. pratensis* to be the cause of root rot of narcissi and strawberries in British Columbia. The evidence submitted here points out that raspberries may also be attacked by this nematode.

*Conclusions Based on Microscopic Examination and Inoculations*

From the above evidence it can be confidently adduced that under certain conditions the roots of raspberries are subject to attack by the following fungi: *Coniothyrium Fuckelii*, *Cylindrocarpon radicicola*, *Cylindrocladium* sp., *Rhizoctonia Solani*, *Rhizoctonia* sp.,(orchid type), *Fusarium* sp. (*F. orthoceras?*), *Pythium* spp., *Asterocystis* sp., and the so-called "mycorrhizal" fungus. Also, since nematodes, especially *A. pratensis*, were unusually abundant in diseased roots from certain districts, though absent in others, *A. pratensis* must be considered as a factor of considerable importance in the root-rot complex.

## Discussion

The special methods recently applied to strawberries by Berkeley and Lauder-Thomson (2), Hildebrand and Koch (19) and Truscott (30), have demonstrated that the roots of raspberries may be attacked by various fungi and nematodes resulting in lesions on roots, and rotting of root tissue. If a high percentage of the roots of a plant are thus affected, its growth of necessity must be greatly reduced, or in severe cases, the plant may be killed.

It is interesting to note that the symptoms of strawberry root rot as described by Berkeley and Lauder-Thomson (2) and Strong and Strong (29) are similar in many respects to those associated with the trouble suspected of being raspberry root rot. In both cases, affected plantations present an uneven and patchy appearance due to the small size of diseased plants and also because of the gaps caused by the death of severely affected plants (Plate I, Figs. 1 and 2). Likewise the roots of both strawberry and raspberry plants show (i) definite black lesions on the newer roots (Plate IV, Fig. 4), (ii) blackened and dead roots, and (iii) a noticeable lack of fibrous roots.

It is of particular interest that seven of eleven fungi associated with strawberry root rot are capable of parasitizing the roots of raspberry. Also microscopic examination has shown that the "phycomycetous mycorrhizal" fungus, *Asterocystis* and nematodes are present in lesions on roots of raspberries as well as in lesions on roots of strawberries affected with root rot.

Evidence has also been submitted which points out that the fungus and nematode complex is not necessarily the same in all soils, as is indicated by the absence of nematodes in one case (Farm P) and their presence in abundance in another (Farm H). Likewise *Asterocystis* was not observed in roots from some districts, while in others it was present in abundance. Moreover, it may well be that there is a seasonal variation in the same soil, as has been recorded by Berkeley and Lauder-Thomson (2) and Hildebrand (18) for strawberry root rot.

Though the evidence submitted herein indicates that raspberries, particularly in British Columbia, may be attacked by root rots, the importance of predisposing factors must not be overlooked. It is generally recognized that plants in a weakened condition from any cause are more subject to disease than plants growing under a favorable environment. For example, Vanterpool (34) has shown that browning root rot of wheat is principally confined to the wheat crop following fallow, and he concludes that "unbalanced available phosphorus and nitrate nitrogen, or perhaps in some instances low available phosphorus alone, is most likely concerned in predisposing the seedlings, or rendering them liable to fungal attack". Dickson (11) has shown that early sowings of wheat in a dry and warm soil were more severely affected with seedling blight than were later sowings in a cool and moist soil. H. Bockmann (4) reports that the blackleg form of foot rot of wheat in the Kiel district, Germany, occurs chiefly on soils of moderate or poor quality, following barley, and to a lesser extent, wheat and rye. According to Sideris and Paxton (28), excess moisture favors Pythium root rot of pineapple. Flor (14) has demonstrated that Pythium root rot of sugar cane was severe only in soils with a water content greater than 50% of the moisture-holding capacity. In Hawaii Heck (17) has demonstrated that soil fertility plays an important role in connection with root rot of sugar cane caused by *Pythium aphanidermatum*. He found that a low phosphorus and high mineral nitrogen content are features of the soils in which root rot is prevalent. Samuel (24) observed that the roots of oats growing in soil deficient in available manganese were heavily infected with an endophytic fungus, whereas roots in normal soils were not so infected. Reed and Fremont (22) in their studies with mycorrhiza of citrus trees found that "roots growing in soils that had received no fertilizers during the preceding seven years had little power to resist invasion or to digest the intracellular mycelium. The mycelium seemed to grow as a pure parasite". On the other hand "roots growing in soils which had annually received application of cover crops and stable manure appeared to develop a definite resistance to the invading fungus". Harris (15) has recently shown that many of the raspberry soils in the coastal areas of British Columbia are

low in one or more of the common nutrient elements, nitrogen, phosphorus, lime and potash, a condition which would undoubtedly tend to render plants more susceptible to attack by parasitic soil fungi. It may well be, therefore, that deficiencies of soil nutrients, or unbalanced nutrients may predispose raspberries to root rot in certain districts in British Columbia.

In the above-mentioned root rots, though the importance of environment and nutrition is recognized, the diseases are nevertheless considered to be parasitic in nature. As Dickson (12) states "the parasite rightly is considered the primary cause of the parasitic disease in plants. Yet the progress of investigations is making more evident each year, the conclusion that in practically all cases the serious development of the disease is conditioned upon certain environmental factors". Again, Vanterpool (34) in his recent paper on browning root rot of wheat rightly points out that "it must not be inferred from the results of chemical soil analysis and fertilizer experiments that the disease is due merely to nutritional disturbances" since *Pythium* sp. has been demonstrated as the primary cause, though it is recognized that unbalanced nutrients may predispose the seedlings to attack.

Similarly, it has been demonstrated (this paper) that raspberry roots may be parasitized by several different soil organisms resulting in destruction of root tissue, and that therefore raspberries under certain conditions may be subject to parasitic root rots. Likewise, it is recognized that the prevalence of raspberry root rot may in no small degree depend on unbalanced nutrition, deficiencies of mineral elements, toxins or other predisposing and important conditioning factors.

## Acknowledgment

The author gratefully acknowledges his indebtedness to Mr. J. J. Woods, Assistant Superintendent, Dominion Experimental Farm, Agassiz, B.C., for supplying naturally affected raspberry plants, as well as plants which had been grown at Agassiz in sterilized and non-sterilized soil from Farms P, H and D. He is likewise in debt to Mr. Woods for the photographs used in Plates I and II, and for several samples of soil in which raspberries had "failed". It would have been impossible to carry out this preliminary study on raspberry root rot without the willing co-operation so kindly extended to the author by Mr. Woods.

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## FURTHER STUDIES ON *FASCIOLOIDES MAGNA* (BASSI, 1875) WARD, 1917, AS A PARASITE OF RUMINANTS[1]

BY W. E. SWALES[2]

### Abstract

Comparative studies have been made on the tissue reactions in fascioloidiasis magna in *Bos taurus*, *Bison bison*, *B. taurus* × *B. bison*, *Cervus canadensis*, *Odocoileus virginianus* and *Ovis aries*. Data have been obtained that support the hypothesis that the Cervidae are normal hosts in the trematode's life-cycle, that the large Bovidae do not act in this capacity and that *Ovis aries* may act as a definitive host but is severely injured by even light infestations. The cellular bases of the defence reactions in the above ruminants are recorded.

Since the publication by the author (10) on the life cycle of *F. magna* and on the preliminary findings in regard to the histopathology of fascioloidiasis magna, further work has been done concerning the parasite in the final host. Krull (7), in a series of experiments involving the experimental infestation of rabbits, sheep, cattle and a pig, with encysted cercariae from *Pseudosuccinea columella*, has corroborated and extended the preliminary observations. In view of Krull's work it seems advisable at this time to present the results of a series of studies made in an attempt to verify and explain the abnormal host-parasite relationships from immunological and histopathological points of view.

As previously shown, the parasite is very pathogenic in *Ovis aries* owing to its almost unchecked migration in the liver tissue. In large Bovidae, *viz.*, *Bos taurus*, *Bison bison*, *Bos grunniens* and *B. taurus* × *B. bison*, the parasite is completely enclosed in a fibrous cyst before it reaches sexual maturity, hence the life cycle is interrupted and these ruminants are unable to act as normal definitive hosts. Also the early cessation of migration of the parasite in large Bovidae prevents undue tissue destruction, resulting in a comparatively low pathogenicity.

In the Cervidae studied, *viz.*, *Cervus canadensis*, *Odocoileus virginianus*, and in other species of Cervidae observed by other workers and myself, the parasite is able to migrate in the liver tissue for some distance before becoming encapsulated. It then becomes enclosed in an adventitious fibrous cavity which differs from the cyst formed in large Bovidae in that it is comparatively thin-walled and most of the intercepted bile ducts are not occluded. These bile ducts then become afferent and efferent ducts in relation to the cavity, the

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former carrying bile from the liver tissue and the latter carrying away the ova and vomitus of the trematode into the hepatic duct, whence they reach the alimentary canal of the host. Thus in Cervidae a host-parasite balance is reached, the parasite being able to continue the life cycle, and the host is not unduly injured by any but very heavy infestations.

The above hypothesis needs the support of further observations upon numerous infested specimens of *Bos taurus*, even though the published works of Krull and the writer at least appear to indicate the rule. In addition to these data are the following observations made upon the sheep (*O. aries*, No. 3) which was still living at the time of my previous publication, and upon an experimentally infested calf (*Bos taurus*).

The sheep (Plate I, Fig. 1) was killed and injected on the 206th day of infestation; at that time it appeared to be near death from fascioloidiasis magna, being extremely cachectic and unable to stand. Eighteen weekly fecal examinations made by a technical assistant failed to reveal the presence of ova of *F. magna*. The autopsy revealed a liver of normal size which was firmly adherent to the diaphragm. Allowing for the change due to the injected fixative, the organ was pale in color, but several areas showed small pigmented spots. An abscess-like projection was present on the left side near the impression of the vena cava. The gall bladder was distended and contained numerous ova of *F. magna*. The negative results of the fecal examinations cannot be explained. One large mature trematode was recovered from a cavity under the aforementioned projection, and a small immature specimen was found in the liver tissue beneath the abomasal impression. A gross section of the liver revealed extensive damage to the tissue of the right lobe brought about by the migration of the immature parasite (Plate II, Fig. 1). The cut surface of the lobe revealed 32 cross sections of a migration route, which was in the form of a convoluted channel at the end of which the trematode was found.

The calf, which had been experimentally exposed to infestation over a period of 141 days, having ingested a total of 180 metacercariae (cercariae ex *Fossaria parva*), was slaughtered and autopsied on the 261st day following the first exposure, which was also the 120th day after ingestion of the last lot of metacercariae. This animal had remained in fair condition throughout the experiment, although gain in weight may have been somewhat retarded. Differential leucocyte counts were made from the peripheral blood on 12 successive days prior to slaughter. These counts are given in Table I.

The high eosinophila shown by these counts is remarkable. This feature, combined with high lymphocyte, low neutrophile, and normal monocyte counts is typical of the picture seen in the healing phase of a septicaemia, and appears to indicate repair of tissue damage.

The autopsy revealed the liver to be heavily infested with *F. magna*. The surface of the organ was irregular and many areas showed the black pigmentation as described by other workers from livers of *Bos taurus* naturally infested with the parasite. The lymph glands of the liver were black in color

TABLE I

| —— | Neutrophiles, % | Lymphocytes, % | Monocytes, % | Eosinophiles, % | Basophiles, % |
|---|---|---|---|---|---|
| 1 | 23.5 | 38.0 | 12.5 | 25.5 | 0.5 |
| 2 | 16.0 | 52.5 | 8.0 | 23.0 | 0.5 |
| 3 | 11.0 | 47.5 | 10.0 | 31.0 | 0.5 |
| 4 | 11.5 | 60.5 | 13.5 | 13.0 | 1.5 |
| 5 | 20.0 | 47.5 | 11.0 | 21.5 | 0 |
| 6 | 18.0 | 47.0 | 10.5 | 24.5 | 0 |
| 7 | 9.5 | 52.0 | 9.5 | 28.5 | 1.0 |
| 8 | 17.5 | 47.5 | 10.0 | 25.0 | 0 |
| 9 | 15.0 | 50.0 | 7.0 | 27.0 | 1.0 |
| 10 | 15.5 | 53.0 | 11.0 | 20.5 | 0 |
| 11 | 13.0 | 47.5 | 9.0 | 29.0 | 1.5 |
| 12 | 11.0 | 52.0 | 6.5 | 30.5 | 0 |
| Average | 15.1 | 49.55 | 9.85 | 25.27 | 0.46 |

and the omentum was spotted with the pigment (Plate I, Fig. 4). The gall bladder and hepatic duct did not contain any ova of *F. magna*. A single cross section through the greater plane of the organ disclosed ten immature trematodes. Subsequent examination revealed the presence of several more specimens, two of which were mature. Owing to the fact that a large part of this liver was retained as a museum specimen, the total number of trematodes present was undetermined. This experimental animal thus added strength to the hypothesis that *Bos taurus* cannot act as a normal definitive host for *F. magna*, and provided tissue specimens for histopathological studies.

Microscopical examination of the feces of eight cattle (*B. taurus*), which ranged over an area from which several other animals had been found on autopsy to be infested, did not reveal the presence of trematode ova. In this enclosure one of the intermediate hosts was present in large numbers and had been exposed to the feces of infested Cervidae. The above-mentioned findings merely add weight to the hypothesis that Bovidae are not normal definitive hosts.

It can be stated quite definitely at this time that there are three distinct defence reactions to invasion by *F. magna*, as seen in the large Bovidae, the Cervidae, and in *O. aries*. These reactions are degrees of vital resistance to the invasion, considered from the point of view of the parasite. The large Bovidae are completely resistant, the Cervidae are tolerant and *O. aries* is partially resistant to *F. magna*, these definitions being made with the understanding that the phenomena are considered as degrees of resistance in so far as the normal life of the parasite is concerned, and that they do not refer to the host's immunity to fascioloidiasis magna.

It was seen that a celluar basis existed for each degree of resistance, and that these bases differed from one another in a quantitative and also, to a lesser extent, in a qualitative way. A study of the structure of the "defence reactions" was made in order to record the active cells in each basis.

## The Cellular Bases of the Defence Reactions

The following observations are based upon studies of the preparations from lesions in *Cervus canadensis*, *Odocoileus virginianus*, *Bos taurus*, *Bison bison*, *B. taurus* × *B. bison* and *Ovis aries*. The tissues were fixed in formalin and the blocks were prepared by standard methods, butyl alcohol being used as the intermediary between alcohol and wax. Sections were cut from 8-10$\mu$ in thickness, depending on the tissue. Two staining methods were used on each specimen, eosin-haematoxylin for the study of cellular infiltrations and Van Gieson's picric-acid fuchsin in conjunction with Harris' aqueous haematoxylin for demonstrating fibrous tissue structures.

The lesions in *C. canadensis* were collected from two aged males, a yearling male, and a three-year-old male, which were parasitized by 14, 40, 15 and 18 trematodes respectively. This material included cavities containing mature trematodes, a young trematode migrating through the tissue, a young trematode becoming encapsulated, the walls of a closed cyst, the path of migration of a young trematode, a fibrous cavity containing an immature specimen, an enlarged bile duct containing one trematode, and portions of liver from between a lesion and the hilum hepatis.

The tissue from *O. virginianus* represented the cyst wall and surrounding parts of lesions containing large mature trematodes.

In addition to the tissue of *B. bison* previously studied (10), tissues from four more of these animals were prepared. These preparations represented lesions containing mature trematodes and showing surrounding liver lobules, tissue containing small immature trematodes partly encapsulated, pigmented tissue and portions of liver from around the lesions.

Tissues from two livers of naturally infested *B. taurus* × *B. bison* were prepared; these were prepared as 15 sections through lesions containing mature and dead trematodes, and sections of surrounding liver tissue. From the liver of the experimentally infested *B. taurus*, 11 preparations of lesions and surrounding tissue were obtained. These were prepared for comparison between this animal and the naturally infested Bovidae, and to show lesions caused by immature parasites.

The livers of three experimentally infested sheep (*O. aries*) were studied. Twenty-nine sections were prepared from the channels of migration, the migrating trematodes, the walls of a large cavity which contained a mature parasite, liver tissue surrounding lesions, and tissue some distance from microscopic lesions.

### Cervidae

#### 1. *Complete Cavities in C. canadensis*

The wall of the cavity is composed of two layers—a richly cellular inner layer of fibrous tissue and a comparatively acellular outer layer of denser fibrils. These layers are separated by a fairly well defined line of demarcation, made by a sudden decrease in the number of cells present in the tissue.

The cellular layer in the specimens examined is from 0.094 to 0.312 mm. and the outer layer from 0.625 to 1.4 mm. in thickness; these figures represent the measurements of what the writer considers to be typical specimens, although it is probable that all lesions in *C. canadensis* will not fall within the above ranges. The cellular layer is composed of fine collagen fibrils and fibroblasts, both phagocytic and non-phagocytic mononuclear exudate cells, eosinophiles, and a very few polymorphonuclear leucocytes. From counts of two areas on each of five preparations of the cellular layers from different lesions the following percentages of the cells were estimated to the nearest 1%:

| | |
|---|---|
| Polyblasts | 68 to 80%; average 74% |
| Fibroblasts | 16 to 24%; average 21% |
| Eosinophiles | 1 to 7%; average 4% |
| Polymorphonuclear leucocytes | 0 to 1%; average 1% |

The above counts were made from lesions which were judged to be typical for this host; about one thousand cells were counted in each area. Adherent to the inner surface of the wall are a few erythrocytes and particles of pigmented detritus.

The more fibrous layer of the wall is composed chiefly of collagen fibrils which gradually increase in density towards the outer edge of the wall. Fibroblasts are interspersed in the fibrils but become less numerous towards the outer edge; the organization of fibrous tissue becomes more complete towards the outer edge, but completely acellular tissue has not been seen in this host.

As previously reported, many of the small bile ducts, intercepted in their course by the formation of the capsule, have not been occluded by the organization of the wall and as many as 16 are seen in a single cross section, some containing ova and detritus. The wall has a blood-vascular system; in most preparations the number of cross sections of vessels was found to vary between 200 and 300, including small capillaries.

The columnar epithelium of the bile ducts is similar in appearance to that of the main ducts in fascioliasis hepatica. The epithelial cells have proliferated, forming assemblages of cells which project into the surrounding tissue in forms similar to foreign body giant cells. Although this formation may be seen in large normal ducts it is not present in normal ducts of similar size to those in a lesion of fascioloidiasis. At the point where a duct enters or leaves the cavity these cells may be seen on the inner surface of the wall adjoining the opening.

The lobules in immediate contact with the fibrous wall are composed of normal liver cells but scattered throughout the chains are eosinophiles, which number 50-100 in each lobule. In an average section several portal canals show the presence of an abnormal amount of fibrous connective tissue. In these inter-lobular canals, the bile duct is sometimes occluded and particles of pigmented detritus are present. These particles compose the black spots seen macroscopically in the tissue of an infested liver. The interlobular ducts,

owing to their small size, are unable to convey ova of *F. magna*, but they apparently attempt to carry detritus from the lesion; this detritus causes a low-grade inflammatory process leading to the formation of fibrous tissue and occlusion of the canal, as would any foreign body.

2. *The Tissue Surrounding a Migrating Trematode in C. canadensis*

The cellular reaction of *Cervus canadensis* to the migrating trematode has been studied in specimens in which the parasite is from 1.3–1.7 cm. in length. The tissue in contact with the trematode is similar in general appearance to the cellular layer of the wall of a well formed cavity, but numerous erythrocytes and particles of pigmented detritus are intermingled in the tissue. The portion of the lesion in contact with the anterior parts of the migrating parasite is in the form of a very thin layer of protective cells; these cells are fibroblasts with fine collagen fibrils, eosinophiles, mononuclear exudate cells (phagocytic and non-phagocytic) and polymorphonuclear leucocytes. The fibroblasts are numerous and the polymorphonuclear leucocytes are very few in number. The eosinophiles are more numerous than in established lesions, and tend to congregate in clumps near the normal liver cells. Towards the posterior part of the parasite the protective layer becomes thicker, but the only noticeable change in its cellular constitution is a proportionate increase in the number of fibroblasts. In the channel behind the parasite there is a mass of erythrocytes, pigment and plasma. The liver tissue immediately surrounding the migration channels is damaged; this damage is seen as displacement of liver cells in an area of two lobules on each side; the cells have been forced out of their typical radiating chain formation, the chains being parallel to the channel and the cells slightly atrophied. The nuclei of the cells appear to be normal and it is probable that complete regeneration of the lobules follows the cessation of the pressure of infiltration and organization of fibrous tissue.

The cellular elements were counted in several fields of the tissue showing defence reaction around the parasite and the cellular layer was found to be constituted approximately as follows:

| | |
|---|---|
| Polyblasts | 70–72% |
| Fibroblasts | 16–20% |
| Eosinophiles | 8–13% |
| Polymorphonuclear leucocytes | 0– 1% |

Owing to the difficulty in seeing the poorly staining cells in the masses of detritus particles, the above-mentioned percentages are not as reliable as those counted in the cellular layers of established and clear lesions.

3. *Complete Cavities in O. virginianus*

The wall of the capsule is essentially the same as it is in the complete cavities in *C. canadensis*. In the available specimens the outer layer is less cellular and the fibres of connective tissue are more dense. The cellular inner layer lacks the eosinophiles noted in the former host, this possibly being due to

the age of the lesion. In other respects the proportions of cellular elements are very similar.

The wall includes a similar blood-vascular system and the bile ducts intercepted by the cavity are open and are active in removing detritus and ova.

The lobules immediately surrounding the lesion are normal and do not show specks of pigmentation. Thus the portal canals are normal and there is no evidence of a continued tissue reaction to the egress of waste products by way of the ducts. On the whole, *O. virginianus* appears to be at least as tolerant to *F. magna* as is *C. canadensis*.

## Large Bovidae

### 1. *Lesions Containing Mature Parasites in Bison bison*

All these lesions have been found to be complete cysts with thick walls of dense fibrous tissue. The walls are very irregular in outline and include masses of detritus and ova (Plate II, Fig. 2). The outer fibrous layer projects into the surrounding liver tissue and in some cases isolates islands of liver cells. The masses of ova and detritus are always surrounded by a cellular layer of tissue which is composed of very variable proportions of polyblasts (chiefly phagocytic mononuclear exudate cells) and fibroblasts. These layers have not been found to include polymorphonuclear leucocytes.

The wall of the true cyst is not so clearly defined as is the wall of the cavity in Cervidae, owing to the above inclusions. However, it is distinctly divided into a cellular and a comparatively acellular layer. Both layers are difficult to describe owing to the extremely variable thickness of the cellular layer and the numerous inclusions and consequent irregularities in the fibrous layer. All bile ducts in and approximate to the lesion are completely occluded by extensive fibrosis; the sites of former ducts are marked by prolonged areas of fibrosis surrounding detritus. A blood-vascular system is present in the fibrous layer of the wall, the vessels being present chiefly in the part approximating the liver tissue.

Fibrous connective tissue is continually being formed on the inner surfaces of the cavity; this fact is shown by the large numbers of ova in the wall which are seen in increasing numbers towards the inner surface but are more firmly encapsulated towards the outer surface. In one lesion, which was sectioned in several places, more than 4,000 ova were present in the wall in each section of ten microns; this indicated the great number of ova present in the wall which enclosed only two trematodes. This fact shows that encroachment of fibrous tissue had been in progress for a considerable time even after the parasites had been completely encapsulated and all bile ducts occluded. This continued fibrous tissue response to the presence of the parasite constitutes the most spectacular difference between the defence reactions of large Bovidae and those of the Cervidae to *F. magna* and its products. The ova in the deeper layers of the wall are more free from detritus owing to phagocytosis having taken place.

The elements in the clearer areas of the cellular layer in the lesions in *B. bison* were found to be in the following approximate proportions:

| | |
|---|---|
| Polyblasts | 75% |
| Fibroblasts | 24% |
| Eosinophiles | 1% |
| Polymorphonuclear leucocytes | 0 |

In typical lesions there are isolated groups of cells which are purely eosinophiles. These groups have not been found in the inner cellular layer but are always approximating areas in which there are numerous small particles of detritus. This detritus, which is undoubtedly the black vomitus of the trematodes, would attract only phagocytic cells and it appears that the function of the eosinophiles in this position is purely phagocytosis.

A section through an entire lesion containing mature trematodes in *B. bison* shows a conglomeration of local cellular reactions amid irregular masses of dense fibrous connective tissue. The space in the actual cavity not taken up by the bodies of the trematodes is filled with a mass of vomitus, polyblasts and a few eosinophiles.

The liver tissue surrounding the lesion is normal in appearance, although in some islands that are surrounded by fibrous tissue the radiating chain-like formation of the cells is disrupted, even although the cells themselves appear normal.

The occluded ducts leading from the lesion towards the hilum hepatis are marked by lines of fibrosis. They are completely occluded for some distance after leaving the lesion and in cross section their appearance is essentially the same as the one shown in Plate III, Fig. 5.

Lesions caused by migrating trematodes or those containing sexually immature specimens have not been found in *B. bison*. Large egg-like masses of dense fibrous tissue are common. These masses could not be readily sectioned; they were surrounded by normal liver tissue and did not contain particles of pigmented detritus.

2. *Lesions Containing Mature Parasites in B. bison* × *B. taurus*

Large fibrosed lesions, similar to those collected from *B. bison*, were the only ones available in the material from several specimens of this hybrid host. The parasites were likewise completely enclosed in fibrous cysts, the walls again appearing as irregular conglomerations of fibrous tissue and local cellular reactions to the presence of detritus. Several sections of the liver tissue were obtained from between the lesion and the hilum hepatis which showed the complete occlusion of the lumina of bile ducts. In no case has an open duct been found in the lesions from this host. The cysts were filled with detritus and ova and it is of interest to note that the sections of the intestinal caeca of the parasites showed the presence of numerous ova; this showed that the enclosed material in the cyst was ingested in place of normal food.

The walls of the cyst, which measured from 1.0 to 2.3 mm. in total thickness, showed the presence of the cellular and fibrous layers, but as in the case of *B. bison*, they were irregular. The cellular elements showed no differences from those in *B. bison*.

*3. Lesions in Bos taurus*

In this host it was possible to obtain the important lesions caused by the migrating parasites in large Bovidae. The microscopical appearance of the tissue surrounding a migrating specimen is strikingly different from that of a similar lesion in *Cervus canadensis*.

The channel of migration directly behind the parasite is filled with red blood corpuscles and detritus as in the other hosts, but this mass is surrounded by a dense layer of eosinophiles which is from 0.8 mm. to 1.3 mm. in thickness. These cells are seen to be actively phagocytosing particles of the pigmented detritus (Plate III, Fig. 6). As many as 15 particles are seen in the cytoplasm of many of the eosinophiles; other cells can be seen with particles adhering to their surfaces as if the particles had been opsonized. In some cases the eosinophiles appear to be phagocytosing the erythrocytes. This active phagocytosis of inert particles and cells by eosinophiles is in conformity with the observations of Weinberg and Séguin (14, 15). As in sections from *B. bison* the eosinophiles are definitely more numerous around the inert particles than around the parasite and this has led me to believe that the particles of vomitus are strongly eosinotactic, and that phagocytosis is at least the primary function of these cells in fascioloidiasis magna of large Bovidae.

The eosinophile layer is in turn covered by another cellular layer in which numerous fibroblasts and polyblasts are seen in a dense network of fine collagen fibrils. In smaller numbers are eosinophiles, degenerate liver cells and inert particles. In this layer the phagocytic mononuclear exudate cells are active in removing inert particles of detritus.

The liver cells surrounding the above layer are normal in individual appearance, but in some places the cell chains are disorganized. In all lobules within a radius of 1.5–1.75 cm. from the channel fine collagen fibrils are intertwined in the cell chains, and the number of fibroblasts present in the lobules is only slightly less than the number of liver cells. The lumen of the central vein in some lobules is filled with eosinophiles and non-phagocytic polyblasts. The portal canals are involved in the reaction, the connective tissue showing hypertrophy and cellular infiltration.

Sections showing the parasite in the process of becoming encapsulated are essentially the same as above in general appearance. The amount of fibrous tissue is somewhat increased; the collagen fibrils are more numerous and are stronger. Fewer eosinophiles are present, but the phagocytic mononuclear exudate cells appear to be present in similar numbers.

OVIS ARIES

1. *A Lesion Containing a Mature Parasite*

The wall of the cavity is a thin layer of delicate fibrils and fibroblasts; it varies in thickness from 0.4 to 0.94 mm. The fibrosis involves the surrounding liver tissue to some extent by irregular projections of fibrous tissue. The wall is not divided into cellular and comparatively acellular layers; numerous polyblasts and a few polymorphonuclear leucocytes are evenly intermingled with the fibroblasts and fibrils throughout the wall. The weakness of some parts of the wall of the cavity suggests that the trematode might have been able to break through and resume its migration had conditions become unfavorable for its existence in this location.

The route taken by the parasite to the point of encapsulation is seen as a wide channel in which the destroyed tissue has been replaced by loose fibrous tissue. Phagocytosis of the detritus has been completed and nearly all of the cells present in the channel are fibroblasts. For a short distance along the channel is a line of loosely encapsulated ova which had evidently been produced by the trematode at the time it was migrating. The presence of these ova in the channel indicates that the parasite migrated for a period of somewhat more than five months before becoming encapsulated and as the parasite was 206 days old at the time of death it had evidently been in the final lesion for somewhat less than 56 days.

The egress of the ova from the cavity was by way of a small bile duct which leads from a point near the end of the migration channel towards the hilum hepatis. Ova and detritus are present in the lumen of the duct; the epithelial layer is somewhat hypertrophied, and many polyblasts (phagocytic mononuclear exudate cells) are present in its folds.

2. *The Channels Made by Migrating Trematodes in O. aries*

The channels have lumina of varying diameters which are filled with pigmented particles of vomitus, erythrocytes and disintegrating liver cells. Surrounding and intermingled with this material are irregular masses of polyblasts and polymorphonuclear leucocytes, and a few eosinophiles; the cellular infiltration is, however, very sparse when compared with similar lesions in *B. taurus*.

The eosinophiles appear to be phagocytosing small inert particles and the monocytoid polyblasts are removing larger particles. The exudate cells extend into the sides of the channel, only changing their character in the total number of cells present. Outside of this layer the fibroblasts appear with accompanying collagen fibrils; these fibrils are very delicate in comparison with those in the Bovidae and do not appear to have the same power of reparation (Plate II, Fig. 5). On the outer edges of this lesion a few fibrils are seen intertwined in the cell chains of surrounding lobules.

The liver tissue immediately surrounding the migrating parasite is somewhat disorganized; the cell chains in adjacent lobules have been forced out of normal position and lie parallel to the channel. Between the anterior end

of the trematode and the normal liver tissue is a thin layer of detritus similar to that in the channel behind, but there is no evidence of fibrosis as seen in the other hosts. In no case have two parasites been seen migrating together in a single channel.

In the whole of the liver tissue in each of the three sheep studied a severe fatty infiltration has been observed. Even the peripheral portions away from an actual lesion have shown this condition as severely as the tissue seen in Plate III, Fig. 2. Fatty degeneration has not been seen; the nuclei in the affected cells are normal in structure but displaced in position by the fat globules. Tissue taken from the antero-ventral border of one liver showed infiltration with polymorphonuclear leucocytes and polyblasts, as well as the fatty infiltration.

## Discussion of the Tissue Defences in Fascioloidiasis Magna

*Fascioloides magna* migrates through the liver tissue of its definitive host causing destruction of the lobules. Most of this destruction ceases when the tissue reaction of the host causes encapsulation of the parasite by fibrous connective tissue. Thus, the manifestations of fascioloidiasis magna in various ruminants are governed by the following host responses:

(1) The fibrous tissue reaction to the presence and activity of the parasite.
(2) The ability of the phagocytic cells to remove detritus.
(3) The regenerative power of surrounding liver tissue.

It is seen that the defences of different hosts may differ considerably in detail, even though the encapsulation of the parasite is certainly the objective in all cases. The details of defence have been found to be more or less constant within certain groups of ruminants but they differ between these groups. These details have a profound effect upon the life of the parasite as well as being all important in regard to the manifestation of clinical disease.

The excessive formation of fibrous tissue in the Bovidae successfully prevents undue damage to the liver of the host as well as precluding continuance of the life cycle. This power of encapsulating the parasite and its products appears to be accompanied by an exudation of great numbers of eosinophiles, and the collagen fibrils formed are undoubtedly stronger than those in the other host groups. This defence activity is not confined to *F. magna* but is seen in the "pipe-stem" liver condition in fascioliasis hepatica in *Bos taurus*, in the fibrous nodules formed around the remains of the filarial nematode, *Setaria labiato-papillosa;* it is also seen, as noted by Dévé (2) in echinococcosis granulosa. It may also be found that *Onchocerca* spp. are so firmly fibrosed that reproduction of the nematode is checked in these animals.

In fascioloidiasis magna the fibrous tissue reaction in Bovidae appears to be continued, even after the parasite is completely encysted, until the whole lesion is fibrosed and phagocytosis is complete. In arriving at this end the process throughout has been one of chronic inflammation localized around the parasite, its products and migration channel, and also local cirrhosis.

The entirely different pathological processes in *Ovis aries* are characterized by a continued acute inflammatory reaction around the damaged tissues, as judged by the presence of comparatively numerous polymorphonuclear leucocytes and the profound general effect on the liver tissue. The inability of this host to check the migration, and hence continued damage, appears to be due to the weakness of the collagen fibrils, which, in turn, appears to be a characteristic of the host species. The absence of a marked eosinophilia in the specimens observed may possibly be concerned in the unchecked migration of the trematode, or it may be that these leucocytes function only in parasitic diseases when the inflammatory reaction is chronic or productive in type.

The Cervidae appear to be entirely suitable for the role of definitive hosts of *F. magna*, the host reaction to the effects and presence of the parasite being adequate to prevent undue tissue damage but not severe enough to prevent reproduction by completely encysting the organism. This tolerance to the effects of a helminth is observed in other helminthiases, filariases for example, and appears to be a natural characteristic of Cervidae rather than a specific tolerance to *F. magna*.

From the immunological point of view it is suggested that the large Bovidae be considered as naturally resistant to the presence of *F. magna*, to the extent of preventing reproduction. *Ovis aries* is a compatible host, but is also intolerant from the parasite's point of view, in that the result of the parasite's invasion is clinical disease which may preclude reproduction. Cervidae are compatible hosts and are tolerant to the effects of the parasite.

In these studies it was unfortunate that the material was limited insofar as tissue from *Bos taurus* is concerned. However, it is hoped that further work of this nature will be done from the point of view of comparative pathology in the case of other helminthiases caused by parasites that invade parenchymatous tissues.

A great number of animals would have to be studied in order to prove beyond doubt that large Bovidae are unable to act as normal definitive hosts in the life cycle, but data now on hand make it reasonably certain that these animals will not spread the parasite nor will they become infested unless they graze on land occupied by infested Cervidae or *Ovis aries* or both. Sheep should be prevented from grazing on snail infested land occupied by Cervidae, particularly as *O. aries* is the only host observed in which *F. magna* produces severe clinical disease.

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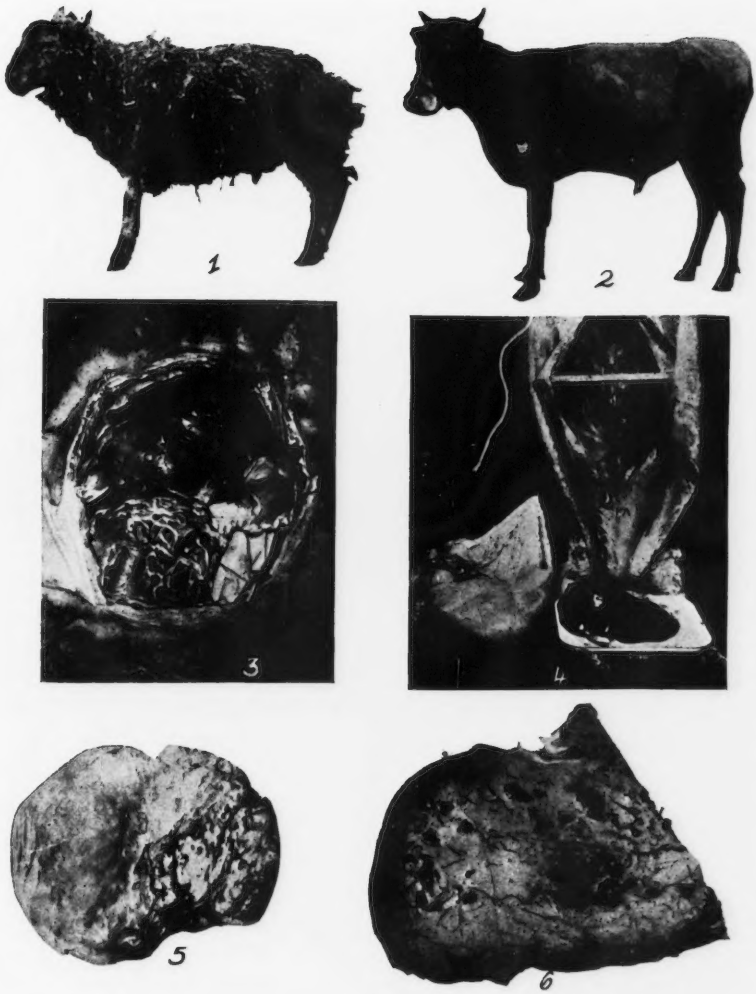


FIG. 1. *Sheep No. 3 on the 204th day of infestation with two trematodes.* 2. *A young bull on the 261st day of infestation with numerous trematodes.* 3. *The appearance of the liver in situ of Sheep No. 3 on the 206th day of infestation.* 4. *The autopsy of the above bull.* 5. *The liver of Sheep No. 2 parasitized by seven trematodes.* 6. *A gross section of a lobe of the liver of the above B. taurus showing the migration channels of nine trematodes.*

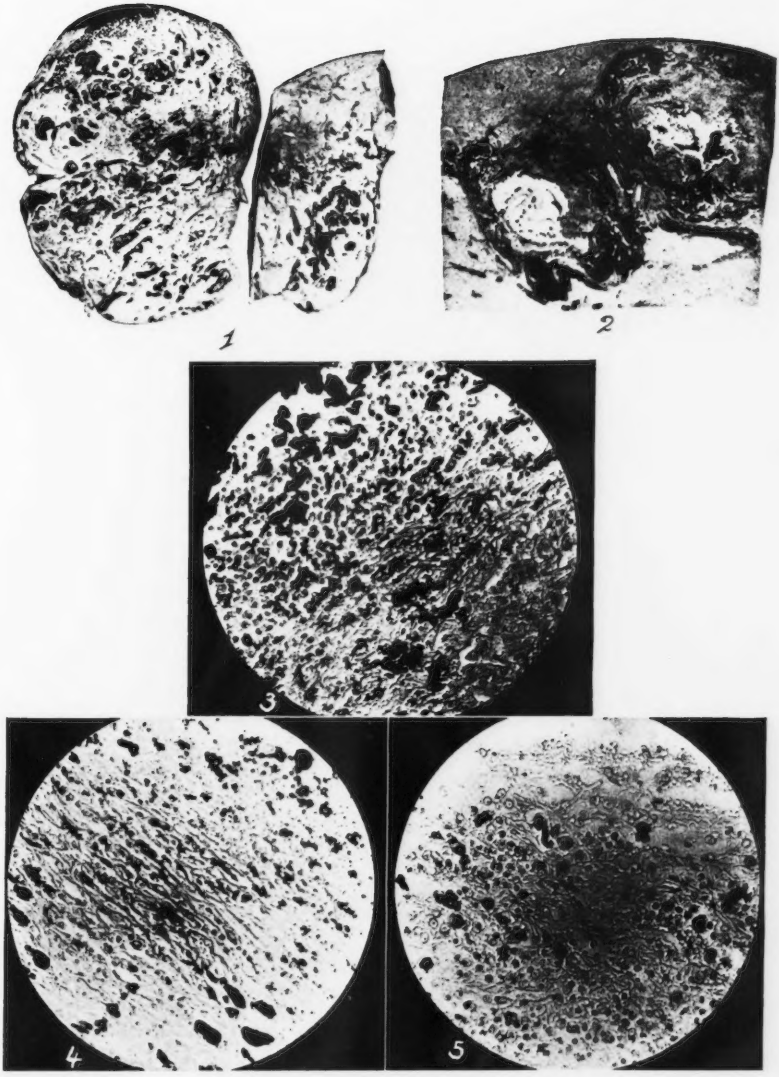


FIG. 1. *Gross sections of the livers of Sheep Nos. 2 and 3. The liver on the left parasitized by seven immature parasites. The portion on the right shows the migration channel of one immature specimen. 2. A section of a typical lesion in B. bison containing two mature trematodes and showing the excessive fibrosis* (× 1·6). *3–5. The fibrous tissue reaction around typical migration channels in B. taurus, C. canadensis and O. aries respectively.*

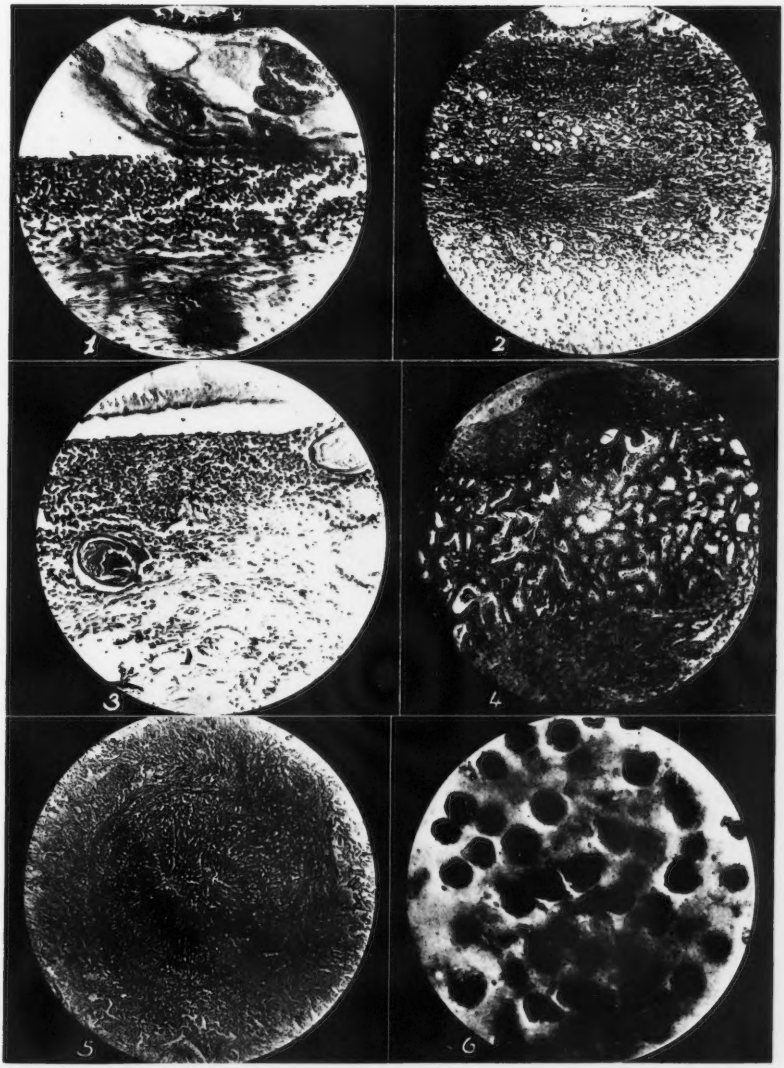


FIG. 1. *A section of a lesion containing a mature parasite in C. canadensis, showing a part of the cavity, the cellular layer, and part of the fibrous layer of the wall (× 97).* 2. *A section through the entire wall of a lesion containing a mature parasite in O. aries (× 83).* 3. *A section of a lesion in B. bison showing the features of Fig. 1 for comparison (× 97).* 4. *The wall of an enlarged hepatic duct which contained a mature parasite in C. canadensis (× 40).* 5. *The occlusion of a bile duct adjacent to a lesion in B. taurus × B. bison (× 84).* 6. *Eosinophiles in a migration channel in B. taurus in the act of phagocytosing granules of detritus.*

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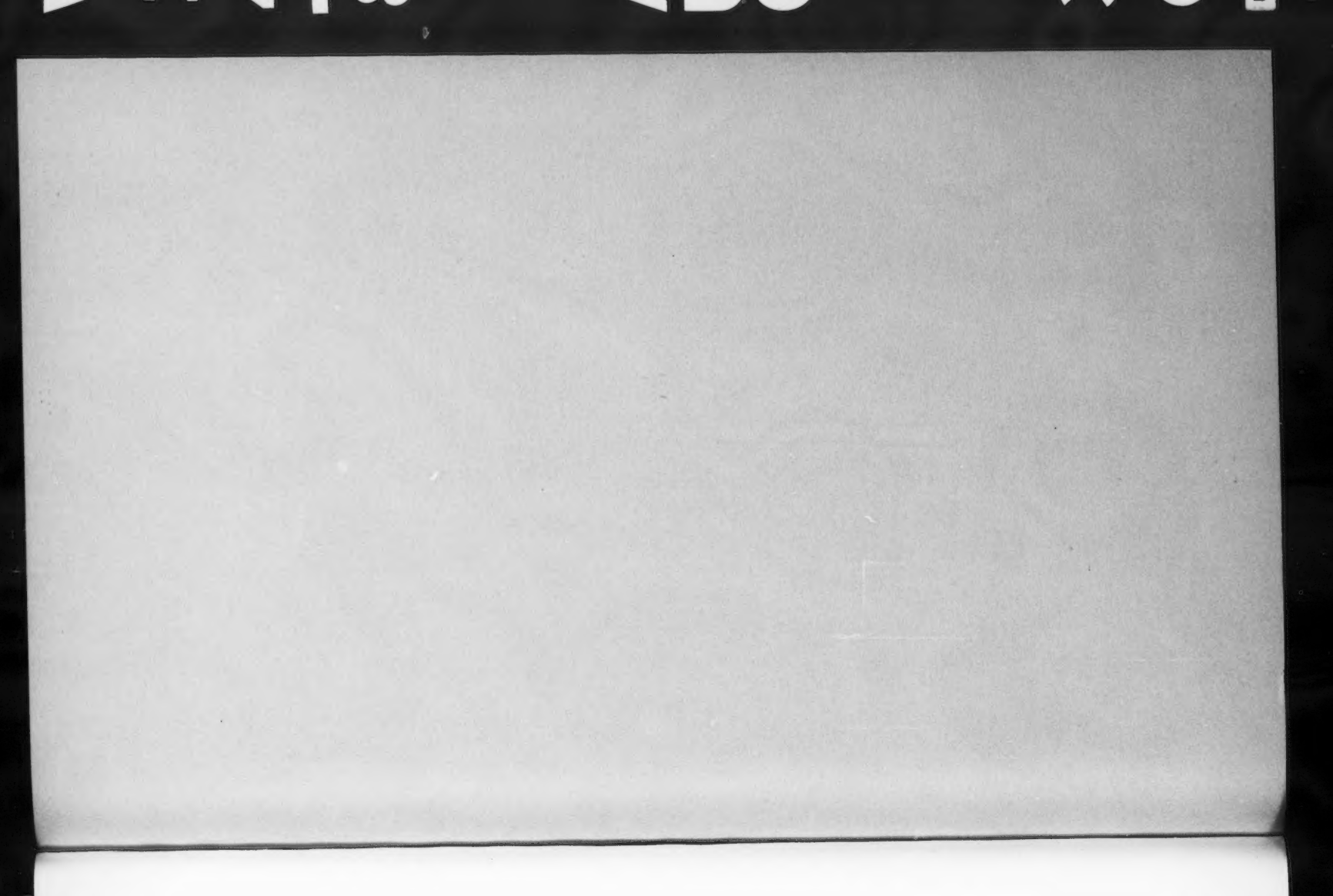

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